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CONTENTS

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PAGE

EDITORIAL—
Thoughts on Applied Science 81

GENERAL ARTICLES—
Ring and Plate Milk Agglutination Tests for Brucellosis in Cattle—A Report from the Agricultural Research Council's Field Station at Compton, the Public Health Laboratory Service and the Ministry of Agriculture's Veterinary Laboratory at Weybridge, on a Combined Testing Programme employing Cattle of Known History
By A. McDiarmid, H. T. Findlay, J. E. Jameson, R. N. Phease, J. H. C. Walker, Lois M. Jones and K. Ogonowski 83

Bovine Cystic Ovarian Disease : An Analysis of 48 cases. Article 2, Part I
By F. L. M. Dawson 96

Studies on Intervertebral Disc Protrusion in the Dog
By L. C. Vaughan 105

Canine Virus Hepatitis in Suckling Puppies
By N. M. Lavine, D.Sc., G. Skulski, M.R.C.V.S., and W. G. Orbell ... 112

REVIEWS 119

NOTICES 122

NEWS 123

NOTICE TO CONTRIBUTORS

Please turn to the inside back cover of this **Journal** *for particulars regarding submission of articles to the Editors.*

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EDITORIAL

THOUGHTS ON APPLIED SCIENCE

FARMERS and veterinary surgeons have always had much in common and their efficiency and success in their respective vocations depends upon factors which are interdependent to a great degree.

They have witnessed remarkable changes in their way of life during the last 20 or 30 years owing to the great advances that have been made in biological science and the application of the new discoveries and procedures to their work in the field.

In the realms of human medicine even greater scientific discoveries have been made and applied to the benefit of patients. Bearing in mind the developments in the medical field, there are some who would say that agriculture and veterinary science have lagged behind in the march of progress. In theory there might be some substance in such a statement but in fact it is not true. There is a significant difference between the practice of medicine and that of agriculture and veterinary science. In the former all efforts must be directed to the preservation of life no matter how hopeless may be the prospect and no matter how useless the individual may be whose life has been prolonged. The patient may be in continuous pain, he may be an encumbrance to his family and cause long periods of anxiety and sorrow and financial distress to those who are near and dear to him. The sacredness of human life over-rules all other mundane factors. Now in the realms of agriculture and veterinary endeavour a more factual and logical attitude is expressed and all procedures, be they of old or new design, have to comply with rigid economic rules. In broad terms, it would be useless to save the life of an animal, no matter how wonderful the methods employed may be, if the cost of the treatment exceeded or even approached to the market value of the patient.

When sentiment is discarded it may be for the general good that academic or scientific steel is tempered by the vigorous fire of economic values. In spite of

this seeming repression no one can deny that great advances and great changes have occurred on the farms and in veterinary practice. These changes have had their effects not only in the actual practice of the arts and the methods employed but even upon the premises at which the work is done. The farms and veterinary surgeons' yards are not as they used to be. Buildings which have slumbered in the sun and served the same purpose for generations have been subjected to drastic alterations. Barns have become byres, stables now house pigs and thatch and tile have been replaced by corrugated iron or asbestos. Horse ponds have been drained, must to the distaste of the local fire authorities, hedges and trees have been razed and the garden countryside turned into a colonial barbed-wired type of ranch in which mechanical juggernauts can work with ease. The ploughman no longer sings his song of joy but bumps along on an iron seat amidst a welter of oily fumes. The veterinary surgeon is not concerned solely with the health of an individual animal and most of his work is now orientated around the mass application of some biological test conceived in the pathological laboratory or in interpreting to the farmer the findings of the research worker on matters appertaining to nutrition, breeding and general health. All these things have to comply to strict economic standards and the men who set the standard are the farmers and practising veterinary surgeons. There can be no doubt that this is a good thing for the academic worker who has no direct contact with the practical world is apt to become enthusiastic and follow a line which may be extremely attractive from the purely scientific point of view but which would fall to the ground when put into field practice, not because of any failing in its own inherent quality and soundness but because it could not stand up to the rigid economic requirements of commercial life.

GENERAL ARTICLES

RING AND PLATE MILK AGGLUTINATION TESTS FOR BRUCELLOSIS IN CATTLE—A REPORT FROM THE AGRICULTURAL RESEARCH COUNCIL'S FIELD STATION AT COMPTON, THE PUBLIC HEALTH LABORATORY SERVICE AND THE MINISTRY OF AGRICULTURE'S VETERINARY LABORATORY AT WEYBRIDGE, ON A COMBINED TESTING PROGRAMME EMPLOYING CATTLE OF KNOWN HISTORY

By A. McDIARMID

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DURING the past few years some difference of opinion has been expressed concerning the respective merits of the Ring and Plate milk agglutination tests for brucellosis. Thus Ogonowski and McDiarmid (1954), working with samples obtained from individual cattle of known history at Compton, considered that the Plate test was preferable to the Ring test for distinguishing between infected and non-infected cattle, thus confirming the view of Blake, Manthei and Goode (1952) in the U.S.A., whereas members of the Public Health Laboratory Service working party on the Ring test found that this was superior for their own particular purpose, namely the screening of milk samples of unknown origin which were to be tested biologically (1956). Because of the possible application of one or other of these tests in an eradication scheme for brucellosis in this country and because of the possible benefit, in so far as biological testing is concerned, in the consequent saving of guinea-pigs by previously detecting which samples should be further examined, it was decided to investigate this matter still further. It appeared feasible to pool our resources and conduct a combined testing programme at different centres using milk samples taken from cows of known brucella status at Compton; these animals were being used for a duration of immunity experiment and for the purpose of that particular investigation groups of cattle, vaccinated in different ways with S.19, were being challenged by the application of 15 or 150 million *Br. abortus* strain 544 by the conjunctival route midway through pregnancy. Some milk samples were also available from normal cows at Compton and others from cattle which, although not infected with brucellosis, where known to react in a non-specific manner to the standard blood sero-agglutination test, employing the standard *Br. abortus* antigen as issued by the Central Veterinary Laboratory at Weybridge.

Accordingly, arrangements were made for the various parties concerned to meet at Weybridge to discuss the techniques to be employed. This meeting took place on

September 27th, 1954; a considerable number of variations in methods were found to exist at the different centres, but eventually a standardised technique for preparing and interpreting the tests was agreed to by those concerned.

Technique and Interpretation of the Tests

Ring Test

It was agreed that, with slight modification, this test could be done according to the original recommendations of the WHO/FAO Committee but that the following points should be stressed:—

(1) The tubes used should be such as to contain the milk in a column 2 cm. high. (2) The volume used should be 1.0 ml. of milk and 0.03 ml. of antigen (to be supplied by Weybridge—same batch to each centre). (3) Mixing should be done by inverting the tube. (4) Incubation—one hour at 37° C. in the incubator. Readings to be taken at more frequent intervals if desired, but final readings at one hour should be recorded for the purpose of comparison between the different laboratories. (5) Readings to be taken as follows:—

+++ Signifies complete agglutination.
++ Signifies 75 per cent agglutination.
+ Signifies 25-50 per cent agglutination.
± This signifies doubtful reaction and must not be grouped with negatives.
N Negative.

Plate Test

The following technique was adopted:—

(1) "Double thickness" grade window glass to be used, not plate glass. (2) Test to be read over a strong light. (3) Quantities: 0.06 ml. milk, 0.02 ml. antigen. (4) It was suggested that 12 tests might be set up at one time. Mixing to be done with tooth-pick type of stick and the mixture spread out to a diameter of about one inch (2.5 cms.). (5) Final reading to be taken at 12 minutes. (6) Readings:—

+++ Signifies complete agglutination.
++ Signifies 75 per cent agglutination.
+ Signifies agglutination from 25-50 per cent.
± Doubtfuls, not to be classed with negatives.
N Negative.

Both the Plate and the Ring tests were to be completed on the day the milks arrived, since no preservative had been added to these samples.

Experimental Procedure

Milk bucket samples were collected from individual cattle exposed to a known experimental brucellosis infection in the isolation units at Compton and these were despatched on the same day at weekly intervals to four Public Health Laboratories, namely Luton, Stafford, Brighton and Winchester, and to the Ministry of Agriculture, Fisheries and Food Central Veterinary Laboratory at Weybridge. Another set of samples was retained for examination at Compton. Variation in the time of arrival at the different centres was unfortunately beyond our control and some delay occurred, especially at holiday weekends with consequent wastage of certain samples. A complete record of all relevant data was kept at Compton. In the first instance we agreed to test at least ten post-partum milk samples from each cow

available for examination after the fourth pregnancy and this has now been accomplished. In addition some vaccinated and control animals in their third lactation were available for testing before drying off and prior to the application of the infective dose in their fourth pregnancy. The animals examined after challenge with either 15 or 150 million *Br. abortus*, were as follows:—

20 non-vaccinated controls, 20 vaccinated at 6 months of age with S.19, 20 vaccinated at 18 months of age with S.19, 20 vaccinated at 6 and 12 months of age with S.19, 20 vaccinated at 6, 12 and 18 months of age with S.19.

With the exception of 5 controls (between 2 and 8 months in calf) all these cows were exposed to infection with *Br. abortus* at about the 5th month of the 4th pregnancy. In all, one hundred cattle were involved—a theoretical total of 1,000 milks over the ten-week period making a total of 6,000 samples examined by each test, a grand total of 12,000 tests.

Results and Discussion

TABLE I

The Results obtained with Individual Milk Samples from Normal Cattle in their 3rd lactation prior to Challenge during the 4th Pregnancy.

Ring

Total : 90 samples 70 negative, 13 '±' } 92%

7 + 8% N.B. obtained from one cow near the 'drying off' stage.

If '±' is considered + the results are:-

78% negative
22% positive

Plate

Total : 90 samples 89 negative, 1 '±' } 100% negative

0 +

If '±' is considered + the results are:-

99% negative
1% positive

Animals which gave '+' or '±' results had shown the intermittent presence of agglutinins in the blood serum for some months previously, but at no time had the titre been greater than 1/20.

TABLE 2

Milk Ring Test :

Results obtained with samples from Normal Cattle in the three dairy herds in which there is no Brucella infection at Compton with indefinite (non-specific) reactions to the Blood Serum Agglutination Test

	Cattle with no reaction to Blood Serum Agglutination Test at Previous Herd Test		Cattle Reacting ($\frac{1}{10}$ or $\frac{1}{20}$) at previous herd blood test	
	Blood	Milk	Blood	Milk
Total: 96 samples from 32 cows	90 N. 94%		27 N. 29%	81 N. 86%
	5 at $\frac{1}{10}$ 5%	All negative	43 $\frac{1}{10}$ 46%	12 '±' 13%
	1 at $\frac{1}{20}$ 1%		24 $\frac{1}{20}$ 25%	1 '+' 1%

N signifies no agglutination

TABLE 3

Ring and Plate Tests : Milk samples from known infected non-vaccinated cattle.

Milk samples were collected at weekly intervals from 20 cattle. Br. abortus had been recovered from every animal and all were positive to the Blood Serum agglutination test. Eighteen of the twenty cows had confirmed infection of the mammary gland.

	if '±' is considered +
Total samples examined Ring (1160), 15% N, 85% +	5% N and 95% +
Total samples examined Plate (1164), 22% N, 78% +	11% N and 89% +

TABLE 4

Samples from cattle vaccinated at 6 months of age.

Milk samples available from 8 cows in the third lactation.

	N	±	+
Ring	121	5	0
Plate	128	1	0

7 of the 8 cows were completely negative to the blood test and 1 animal reacted at $\frac{1}{10}$.

TABLE 5

Ring and Plate Milk Agglutination Tests with milk obtained from Cattle Vaccinated at 6 months of age and failing to resist a subsequent challenge dose with *Br. abortus* in their 4th pregnancy.

No. of cow	Infective Dose	Blood Serum* Agglutination Test	Pooled results of Ring and Plate tests				
				+	±	N	Total no. of samples examined
212	15 Million	1	R	38	8	10	56
			P	19	6	31	56
223		5	R	59	0	0	59
			P	48	6	5	59
300	150 Million	6	R	59	1	0	60
			P	26	4	29	59
316		6	R	19	22	19	60
			P	58	2	0	60
423		4	R	21	18	19	58
			P	6	4	48	58
598		4	R	34	18	6	58
			P	36	8	13	57
629		6	R	60	0	0	60
			P	60	0	0	60
J75		4	R	30	14	3	47
			P	5	10	32	47

* In this and subsequent tables, the titres are expressed as tube numbers Thus
1 signifies 1/10,
2 signifies 1/20, 70% RT+ 57% PT+
3 signifies 1/40 etc. 30% RTN 43% PT+

Apart from minor incidents, the experiment was conducted according to the initial plan; the only serious modification which had to be made was during a rail strike which restricted our activities to the centres nearest to Compton, namely Winchester and Weybridge. The tables 1 to 15 show the pooled results for the different groups obtained at the various centres and Table 16 shows a compilation of data pertaining to the individual results at the different centres. Further detailed individual figures are available on request from Compton.

The general points of interest arising from a consideration of these tables are:—

(1) Both the plate and ring tests were generally negative with milk from normal cattle with the exception of samples from cows approaching the drying-off period. The plate test appeared to give a clearer negative picture than the ring test in this group of non-infected animals (Table 1).

(2) The "±" reaction in milk agglutination tests appeared in many instances to be non-specific in character (Table 2). We have found recently that incubation at 50° C. or 56° C. for one hour eliminates some of these non-specific "±s", an observation that was made in the early work on blood sero-agglutination tests by

TABLE 6

The results of Ring and Plate tests with milk from cattle vaccinated at 6 months of age and proved resistant to a challenge dose of *Br. abortus* in the 4th pregnancy

No. of cow	Blood serum agglutination titre	Infective Dose		Results of Ring and Plate tests over a 10 week period after calving. +	±	N	Total: samples examined from each animal
42	1	15 Million	R	0	0	57	57
			P	0	4	51	55
75	1		R	22	17	10	49
			P	13	5	31	49
201	2		R	1	0	55	56
			P	0	7	48	55
302	3		R	20	23	16	59
			P	7	12	40	59
399	2		R	3	7	45	55
			P	7	4	43	54
499			R	1	0	57	58
			P	2	3	53	58
514	1		R	0	4	50	54
			P	0	1	53	54
638	1		R	1	0	55	56
			P	1	2	53	56
66	N	150 Million	R	2	6	49	57
			P	0	7	50	57
157	3		R	0	5	50	55
			P	0	6	48	54
572	N		R	0	0	54	54
			P	1	2	52	55
578	N		R	4	9	42	55
			P	3	8	44	55

8% positive RT 92% negative RT
6% positive PT 94% negative PT

TABLE 7

Ring and Plate test results with milk samples obtained prior to challenge from cattle vaccinated at 18 months of age and examined towards the end of their third lactation.

Milk samples were available from 8 animals.

	N	±	+
Ring Tests	90	43	194
Plate Tests	239	23	65

TABLE 8
Results of Ring and Plate tests with Milk from cattle vaccinated at 18 months of age and failing to resist a subsequent challenge dose of *Br. abortus* in their 4th pregnancy.

No. of Cow	Blood Serum Titre	Infective Dose	Results of Ring and Plate tests over a 10 week period after calving							
			N		±		+		Total	
			R	P	R	P	R	P	R	P
78	5	25 Million	8	27	14	10	38	22	60	59
368	6		1	1	14	6	45	53	60	60
446	3		33	6	8	7	15	43	56	56
576	7		-	-	3	4	54	53	57	57
309	6	150 Million	12	34	16	10	32	16	60	60
409	7		-	20	-	12	57	25	57	57
445	5		5	28	9	8	45	23	59	59
548	8		Dead							
621	4		-	49	4	7	56	4	60	60
			59	165	68	64	342	239	469	468

Total number of tests 937

Nègre and Raynaud (1911, 1912). Tests at these temperatures will often show a decrease in the intensity of the reaction of milk from known infected cows. Thus a milk showing a reaction of " +++ " at 37° C. might well become " ++ " when tested at 50° C. These observations are perhaps worthy of further investigation, because reduction of the number of " ± s " would correspondingly reduce the number of biological tests required on milk samples submitted to the public health laboratories.

(3) In infected control cattle (Table 3) the individual milk tests were not so accurate as the blood sero-agglutination tests. The Ring test appeared to give better results than the Plate method in this instance; the detection rate of the former varied between 85 per cent and 95 per cent depending on the classification of the " ± s ", whereas the accuracy of the Plate test ranged from 78 per cent to 89 per cent. Thus, as already indicated in Table 1, the Ring test is the more sensitive of the two methods.

(4) In a limited number of eight animals, vaccination at 6 months of age did not affect subsequent milk agglutination tests in the 3rd lactation (Table 4). This confirms our previous experience in earlier lactations (Ogonowski and McDiarmid, 1954). This vaccination performed once in calfhood would not interfere with an eradication programme dependent on the use of the milk agglutina-

tion tests provided the animals were not exposed to a considerable weight of natural infection subsequent to vaccination.

(5) **When animals** vaccinated at 6 months of age were challenged with *Br. abortus* in their fourth pregnancy, those which failed to resist infection gave results which were difficult to interpret (Table 5) whereas most of those which resisted infection were negative to the tests (Table 6).

(6) Table 7 shows the results obtained with samples taken before challenge from cattle vaccinated at 18 months of age. In this group the Plate test again gave more negative results than the Ring test, but the usefulness of both tests was limited because of the high proportion of positive results obtained by both methods. When these cattle were challenged subsequently, those which failed to resist the infection generally gave positive results to both tests but numerous negative tests still occurred and diminished the usefulness of the tests (Table 8). In Table 9, the results of testing milks from cattle known to be resistant to challenge showed that the majority were negative to both tests, but the presence of positives in this group again prevented the test being of much value in distinguishing between the different classes of animals.

TABLE 9

Results of Ring and Plate tests with milk from cattle vaccinated at 18 months of age and proving resistant to a challenge dose of *Br. abortus* in their 4th pregnancy.

No. of Cow	Blood Serum Titre	Infective Dose	Results of Ring and Plate tests over a 10 week period after calving							
			N		±		+		Total	
			R	P	R	P	R	P	R	P
23	4	150 Million	-	35	4	11	53	10	57	56
211	4		47	47	7	6	2	2	56	55
340	4		48	48	8	5	-	2	56	55
351	2		51	54	5	1	1	1	57	56
383	3		55	55	1	1	-	-	56	56
465	4		2	12	8	5	19	11	29	28
74	4	150 Million	47	47	5	2	2	3	54	52
200	2		46	51	6	6	5	-	57	57
373	3		49	48	8	3	-	6	57	57
394	3		50	50	4	2	-	1	54	53
422	3		49	53	6	2	1	-	56	55
			444	500	62	44	83	36	589	580
									Total number of tests	1169

TABLE 10

Ring and Plate test results with milk samples obtained prior to challenge from cattle vaccinated at 6 and 12 months of age and examined towards the end of their third lactation.

Milk samples were available from 8 animals.

	N	±	+
Ring Tests	122	25	120
Plate Tests	208	29	31

TABLE 11

Results of Ring and Plate tests with milk from cattle vaccinated at 6 and 12 months of age and failing to resist a subsequent challenge dose of *Br. abortus* in their 4th pregnancy

No. of Cow	Blood Serum Titre	Infective Dose	Results of Ring and Plate tests over a 10 week period after calving							
			N		±		+		Total	
			R	P	R	P	R	P	R	P
187	7	15 Million	1	2	4	7	55	50	60	59
204	4	15 Million	-	8	-	8	59	42	59	58
228	3	150 Million	53	43	6	8	-	6	59	57
318	6	150 Million	-	-	-	-	57	57	57	57
428	7	150 Million	-	7	-	5	58	47	58	59
			54	60	10	28	229	202	293	290

Total number of tests 583

(7) Table 10 illustrates the results obtained with samples of milk taken during the third lactation from cattle vaccinated twice, at 6 and 12 months of age. The majority were negative to both tests but positive reactions still persisted, particularly in the Ring test group.

When these cattle were exposed to infection later, the majority of those which failed to resist infection (Table 11) gave positive results to both tests, although again several milks were completely negative to both tests. Those which resisted infection (Table 12) gave a somewhat better picture in that the majority of cattle

TABLE 12

Results of Ring and Plate tests with milk from cattle vaccinated at 6 and 12 months of age and proving resistant to a challenge dose of *Br. abortus* in their 4th pregnancy.

No. of Cow	Blood Serum Titre	Infective Dose	Results of Ring and Plate tests over a 10 week period after calving							
			N		±		+		Total	
			R	P	R	P	R	P	R	P
25	2	15 Million	50	46	7	7	-	3	57	56
112	N		50	54	5	3	2	-	57	57
236	1		59	51	-	1	-	5	59	57
258	1		57	42	-	5	-	6	57	53
314	2		47	30	2	7	-	10	49	47
333	3		31	49	20	4	6	3	57	56
374	1		Dead							
612	1		52	50	2	4	-	1	54	55
43	1	150 Million	55	44	-	6	-	5	55	55
103	3		10	46	21	8	26	3	57	57
178	N		42	43	12	4	1	7	55	54
193	3		28	45	15	2	12	7	55	54
233	2		2	47	10	5	43	3	55	55
263	2		36	53	15	2	5	-	56	55
341	3		37	47	13	5	5	3	55	55
			556	647	122	63	100	56	778	766

Total number of tests 1544

TABLE 13

Ring and Plate tests results with milk samples obtained prior to challenge from cattle vaccinated at 6, 12 and 18 months of age and examined towards the end of their third lactation.

Milk samples were available from 7 animals

	N	±	+
Ring test	164	26	91
Plate test	253	17	10

TABLE 14

Results of Ring and Plate tests with milk from cattle vaccinated at 6, 12 and 18 months of age and failing to resist a subsequent challenge dose of *Br. abortus* in their 4th pregnancy.

No. of Cow	Blood Serum Titre	Infective Dose	Results of Ring and Plate tests over a 10 week period after calving							
			N		±		+		Total	
			R	P	R	P	R	P	R	P
11	7	15 Million	17	36	16	12	25	10	58	58
447	6		1	24	10	10	47	22	58	56
65	6	150 Million	1	4	8	3	45	46	54	53
253	8		-	10	-	6	58	42	58	58
285	9		-	-	7	-	45	52	52	52
290	4		-	6	-	4	60	50	60	60
387	6		-	35	8	11	47	9	55	55
564	6		1	38	3	9	56	13	60	60
			20	153	52	55	383	244	455	452

Total number of tests : 907

were negative to both tests, but the proportion of milks still reacting to the tests was sufficient to cause complications in an individual milk testing programme.

(8) Table 13 depicts the results obtained with milk samples taken before challenge from cattle vaccinated at 6, 12 and 18 months of age. It will be seen from this table that triple vaccination with S.19 resulted in a proportion of vaccinated animals reacting to both the Ring and Plate tests even in their third lactation, although the majority were negative to both tests. When cattle vaccinated in this way were exposed to experimental brucellosis, those which failed to resist infection (Table 14) showed a high proportion of reactors compared with those which resisted the subsequent infection (Table 15). The number of reactors, however, in the latter group was still sufficiently large to make the interpretation of the test, in so far as the separation of the cattle into infected and non-infected categories was concerned, extremely difficult. Table 16, kindly prepared by Dr. R. C. Campbell of the Agricultural Research Council's statistical group, depicts the proportion of milks judged to be positive by the Ring and Plate tests at the different centres. There is no doubt that, when the evidence from the six different centres is considered as a whole, the Ring test is more reliable than the Plate test for detecting infected animals. It is, in fact, the more sensitive test and although the Plate test will give fewer false positives under certain conditions such as in the absence of infection, the Ring test will select a higher proportion of infected animals.

TABLE 15

Results of Ring and Plate test with milk from cattle vaccinated at 6, 12 and 18 months of age and proving resistant to a challenge dose of *Br. abortus* in their 4th pregnancy.

No. of Cow	Blood Serum Titre	Infective Dose	Results of Ring and Plate tests over a 10 week period after calving							
			N		±		+		Total	
			R	P	R	P	R	P	R	P
110	3	15 Million	46	37	9	12	-	6	55	55
138	1		54	43	1	6	1	7	56	56
239	3		46	41	-	3	-	2	46	46
262	1		Dead							
370	3		54	44	1	7	-	3	55	54
436	3		39	46	12	5	4	4	55	55
464	1		52	37	5	10	-	8	57	55
526	3		55	54	-	1	-	-	55	55
73	3	150 Million	41	45	12	6	4	4	57	55
164	3		15	39	22	10	19	7	56	56
272	2		38	49	8	3	8	1	54	53
518	1		55	54	1	2	-	-	56	56
			495	489	71	65	36	42	602	596

Total number of tests : 1198

TABLE 16

The proportion of milks, form cows of known histories, judged to be positive by the Ring and Plate tests at different centres.

Group	Centre													
	Brighton		Compton		Luton		Stafford		Weybridge				Winchester	
									Ogonowski		Jones			
	Ring	Plate	Ring	Plate	Ring	Plate	Ring	Plate	Ring	Plate	Ring	Plate	Ring	Plate
Infected*														
I	0.90	0.56	0.59	0.80	0.77	0.41	0.75	0.41	0.69	0.73	0.89	0.54	0.75	0.62
II	0.91	0.48	0.41	0.77	0.71	0.31	0.69	0.36	0.80	0.63	0.81	0.32	0.70	0.55
III	0.82	0.79	0.73	0.86	0.80	0.40	0.78	0.61	0.70	0.74	0.77	0.60	0.75	0.81
IV	0.91	0.51	0.61	0.67	0.87	0.35	0.80	0.30	0.84	0.64	0.96	0.31	0.82	0.50
V	0.92	0.72	0.72	0.88	0.87	0.61	0.86	0.63	0.85	0.82	0.87	0.72	0.85	0.74
Total	0.90	0.63	0.63	0.81	0.92	0.47	0.80	0.49	0.81	0.75	0.86	0.53	0.79	0.66
Non-infected														
I	0.10	0.02	0.04	0.19	0.11	0.01	0.06	0	0.50	0.35	0.06	0	0.07	0
II	0.18	0.02	0.09	0.21	0.14	0	0.10	0.02	0.60	0.80	0.15	0	0.14	0.03
III	0.18	0.02	0.05	0.29	0.15	0.02	0.07	0.01	0	0	0.18	0	0.12	0.01
IV	0.11	0	0.02	0.29	0.08	0	0.01	0.01	-	-	0.05	0.05	0.06	0
V	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Total	0.14	0.02	0.05	0.24	0.12	0.01	0.06	0.01	0.43	0.37	0.11	0.01	0.10	0.01

* The Roman figures signify the vaccination groups to which the cattle belong.

Thus: I signifies vaccinated once at 6 months of age.
II " " " " " 18 " " "
III " " twice at 6 and 12 months of age.
IV " " three times at 6,12 and 18 months of age.
V " non-vaccinated controls.

‡ Signifies a change of workers during the course of the experiment.

From a consideration of all the available data there appears to be no evidence of any marked differences between the groups vaccinated in different ways when considered in three separate categories, namely, non-challenged, challenged but not infected, challenged and infected. There was a very small proportion of milks positive to these tests in the first class, single vaccination at 6 months of age causing least interference with subsequent milk tests, a substantially higher proportion in the second class and a still higher proportion of milks were positive in the third class. In all classes considerable differences occurred between cows in the proportion of milks positive.

Conclusions

From these results, it may be concluded that the Ring and Plate milk agglutination tests are of no great value in determining the state of immunity of individual cattle vaccinated in different ways with S.19 vaccine before breeding age and subsequently exposed to a known *Br. abortus* infection. Milks from non-infected cows may give a positive response and many samples from known infected cows fail to react to these tests. The Ring test was shown to be more sensitive than the Plate test under the conditions of the present investigation and the vaccination of calves with S.19 on a single occasion at about six months of age, appeared to interfere least with subsequent Ring tests even in the presence of super-imposed infection, provided that the animals in question resisted the challenge dose. Under field conditions the possibility of cattle, vaccinated in this way, being exposed to such a degree of infection would be remote.

Acknowledgement

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BOVINE CYSTIC OVARIAN DISEASE: AN ANALYSIS OF 48 CASES

Article 2

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General Introduction

A REVIEW of this condition (Dawson, 1957) indicated that little work has been done on material of British origin. This material was obtained in 1953-6 from barren dairy cattle discarded from 95 different farms. The animals were kept under close observation for periods averaging four weeks before slaughter, after which an examination of the genitalia was made. This included ærobic cultures on horse-blood agar plates of inocula from the endometrial mucosa; dissection of the organs; fluid permeability tests of the oviducts, and the preparation of sections, stained with hæmatoxylin and eosin from the oviducts, uterus, and in some cases, ovaries. Frequently the status of the herds of origin regarding *Vibrio fœtus* infection was known, and usually individual histories were adequate. No hormone assay work or investigation of pituitary, adrenal or other endocrine organs was made.

Part 1: Clinical-Pathological Aspects

The lack of relevant British material justifies putting on record some detail of the features encountered. It has been possible to augment the general sum of knowledge on certain aspects of the disease. These included the bacteriological and pathological analysis of the uterine pathology; only Garm (1949) carried out a thorough study in this connection, and he felt that the results were atypical, a conclusion confirmed by " synthesis " from other available reports. Hitherto the study of the condition of the cervix had been neglected. A paper, by Vandeplassche (1951) had pointed out the frequent occurrence of " intermediacy "—nymphomania with some masculising changes, and although it was possible to adduce further evidence about this phenomenon, this too was an aspect which requires additional study. The direct and in some cases quantitative study of the behaviour of the endocrine organs involved, other than the ovary was not attempted in this investigation.

Adrenal Virilism

The literary review attempted to justify the findings of *luteal* cysts as indicating the presence of the condition of *virilism*, and showed either on these or better grounds that three authors were agreed on the occurrence of true virilism in about 20 per cent of " cystic " cases; while two other authors stated vaguely that such formed " a small proportion " of the whole. Vandeplassche's (1951) estimate of 5 per cent may have been too low, since it related to those *unaffected* by spaying, but there is reason to suppose that the presence of the ovaries may " modulate " the diseased condition in the adrenals. In the present material there were seven cases or 15 per cent yielding cysts of definitely luteal type. The seventh case showed no other signs of cystic disease. In view of other workers' agreement on 20 per cent, among similar-sized series of cases, the divergency is not without interest and may be proved with

further study to be significant. Table Ia summarising the findings in these cases confirms the basis of selection; since on the evidence of the voice and behaviour two appeared to be typical virilism cases; these criteria could not be judged very well in the next three, for they could only be studied when stalled during the winter. The first five cases show a general agreement on the type of cyst encountered—in three these recurred following rupture—and also in that œstrogen production appeared to be low, directly comparable, as far as can be estimated, with that in Garm's well-investigated virilism case series. Perhaps the most striking feature in Table I is the consistency with which cystic glandular dilatation is found in the endometria of the first four animals. The condition was recognisable in two despite severe chronic inflammatory changes, but the latter may have masked the condition in the last two. Ajello (1947) has described dilatation in a single " luteal cyst " case, but other authors seem to have neglected the uterus simply assuming the occurrence there of a tendency to atrophy.

TABLE I

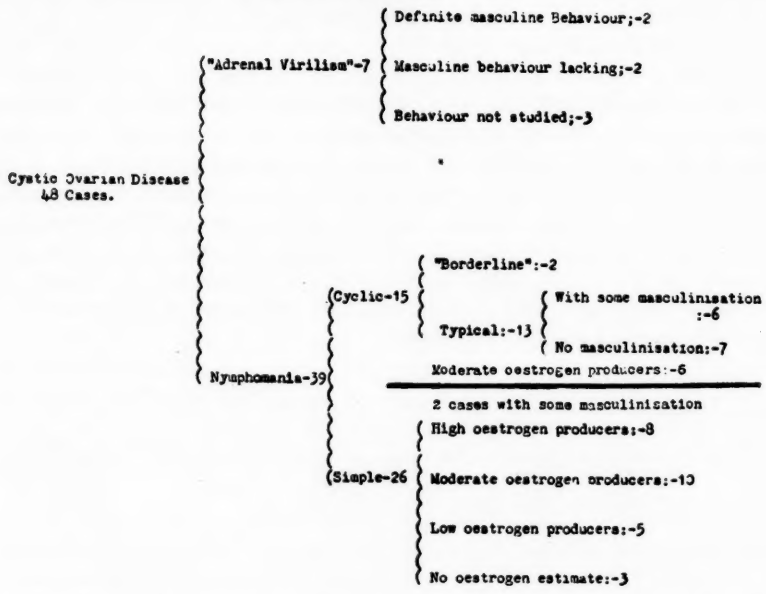
Adrenal Virilism Cases.

Age	Case	Nature of Cysts	Evidence of Oestrogen Production.	State of Endometrium	Voice	Behaviour	Remarks
6	LM.Guernsey	1 1.5 cm, 1 hemisphere solid luteal tissue; + 1 unovulated solid "luteal body" See remarks	Tailhead markedly raised. 2 "follicularcysts" 1.0 & 1 .25 Some evidence of slowed cycle persisting	(C.pyogenes discharge during life). Sterile autopsycultures. "Abscess or cyst" found on dissection. Severe endometritis - no gland tissue in section	Decidedly masculine	Digging with fore-feet.	Large "luteal cysts" ruptured repeatedly during clinical study "typical case"
6½	E.Guernsey	1 2.0 cm & 2 of 1.0 cm All well lined	None. "Masculine atrophied" cervix and vagina	Some 'ghost' gland sites (severe endomet) still show quite convincing pronounced cystic dilatation. uterus much enlarged and wall thin.	?	6 mths. after calving, 3 after service suddenly began behaving like a bull!	"Typical Case"
3½	W.Guernsey	2, 1.25 & 1.0 thinly lined. See also remarks	Vascular congestion of tube ampulla & endometrium.	Severe endometritis with cystic dilatation in less affected patches. Surface congestion.	?	?	A normal cycle gave place to this condition, developed 4 weeks antemortem. Luteal cysts 2.5 & 1.5. ruptured during clinical study.
9	F.Guernsey	1 slightly hollow & 1 cystic, c.l. See also remarks	Nil. except that ovulation took place during clinical study	Sterile. Gross degree of glandular cystic dilatation & hydrometria.	?	?	Luteal cyst 3.0 ruptured at first clinical examination.
8	P.Guernsey	½ from 7 cm downwards lining up to 2 mm thick	Follicular cysts 2, 2.0. Slack cervix, slow persisting cycle. 45 days.	Numerous staphylococci & Bact.coli. 'Ghost' gland sites etc. Evidence severe endometritis. No cystic dilatation	?	?	
3½	D.Friesian	1 1.0 with a luteinised hemisphere, cystic c.l. 10 cystic remnants See remarks.	Regular cycles with normal oestrus signs. 1 tubal ampulla fairly highly vascular.	Sterile. Severe endometritis. 'Ghost' gland sites etc.	Normal	Normal	2 larger luteal cysts ruptured. 2 days ante-mortem A typical borderline case.

The Ovary in " Cyclic " Nymphomania

Within the main large group exhibiting hyperœstrinæmia, there occurs a clear-cut sub-group in which, alongside the development of chronic active follicular cysts, the continuance of some form of regular ovarian cycle with corpus luteum formation was evidenced. In one of Garm's four groups, that exhibiting regular heat, he considered such behaviour by the ovary probable; as did Vandeplassche, who dissected ovaries from four nymphomaniac cows, and found that they contained corpora lutea alongside the cysts. Gruber's (1952) abattoir survey gave a proportion of 3 out of 21 cystic cases with accompanying corpora lutea, and Yamauchi (20) found them in 2 out of 12 sets of ovaries from nymphomaniac cows. One of these cows was " pregnant "!

TABLE Ia.
Summary of findings



The small numbers studied by Gruber and Yamauchi suggest that only Garm's work yielded any indication of the relative incidence of "retained Cycle" cases. He found them to be about one-fourth of the total. In the present series, a similar proportion is noted. These 13 cases seem to be unique in the literature. The clinical studies which were maintained up to the time of slaughter place the cyclic behaviour of the ovaries beyond any reasonable doubt. Only four cases have been selected as the types for analysis in Table II, on the basis that the data concerning them are the most complete. The table shows also the typical co-existent nymphomaniac syndrome, the incidence of relaxed pelvic ligaments (6 of the 13 cases), recalling Garm's figure of 50 per cent for his regular heat animals, and higher than he found for his anœstrous group or for the group with long weak heats. The occurrence of large numbers of small "grape bunch" cysts, alongside the large cysts, in three cases is curious, since according to the literature the grape-bunch ovary is quite uncommon. In two other cases only grape-bunch cysts occurred. These cases are excluded from many of the analyses which follow. The occurrence of masculinisation signs in half the cases is interesting. In over 200 nymphomaniacs, Vandeplassche found 40 per cent exhibiting such intermediacy. The incidence is much lower in the remainder of the present material—i.e., in the simple nymphomaniacs. Well-developed spherical luteal bodies, exhibiting no trace of any ovulation scar, were found in four cases. In a fifth a hollow body was seen and it exhibited a pin-point site from which an ovum may have been shed. It was very different from a normal ovulation scar. Yamauchi (1955) found cysts to react in this same way

to LH, when already their theca interna had partially degenerated. This suggests that in the co-existent cycle nymphomaniac, there is a normal rhythmic liberation of LH/LTH, acting upon follicular cysts, the result of continuous excessive FSH secretion. The evidence in five cases suggested (as for instance where the cystic condition supervened only during a prolonged clinical study) that periods of normal type ovarian activity, or conversely of acyclic " simple " nymphomania, alternated with the co-existence of a cycle accompanying the nymphomania. Two, perhaps three, of the cases suggested that *treatment* restarted rhythmic c.l. formation, while follicular cyst production remained unaffected. In this connection the case yielding empty fœtal membranes in full nymphomania is of interest, and recalls Yamauchi's finding of " pregnancy " in one cow. As Yamauchi did not describe the actual fœtus, this could have been a similar case. If this be so it remains to be proved that pregnancy in nymphomania is possible! One other uterus in this series had an unusual content, 500 ml. of tacky mucus; the tendency to endometrial glandular dilatation appeared less strong than in the " adrenal virilism " group, only seven organs exhibiting such tendency, and in each case to a very limited extent.

TABLE II

Examples of cyclic nymphomaniacs.

Age	Case Identity	Nature of Follicular cysts at post-mortem	Evidence of continuous oestrogen production		Evidence of regular recurrent cycle		Evidence of Masculinisation	State of Endometrium
			General	From Clinical Study	Dissection Findings	From Clinical Study		
4	G.Jersey	1,1.5 cm. & also 1 luteal (encapsular bursitis). 18 small, grape bunch type	Tailhead markedly raised (asymmetry) ligament slack on left side only. Irregular frequent "oestrus"		'Regressor' 1.5 cm. recent remnant. 7 older remnants	Normal cyclic cervico-vaginal changes followed over 19 days.	Nil Luteal cyst atypical, result of encapsulation of ovary through pressure	Much free blood. Slight endometritis. A little glandular dilatation. Large wall abscess.
5	S.Guernsey	2, 2.0 & 1.5 cm	Tailhead somewhat raised	Follicular Cysts present throughout 24 days	Corpus Luteum 2.0. (with abnormal ovulation point) & 5 remnants	Normal cyclic changes followed in cervix and vagina and some phases in ovary	Early in disease course was interested in bulling cows; this ceased after lutermone which may have restored cycle	Fairly normal but a few glands show cystic dilatation
8	Ir.Ayrshire	3, 1.0-2.75 cm and 20 small grape bunch type	Oestrous and service on 2 occasions 4 days apart	Cysts present throughout 21 days. Intermittent non-cyclic oestrous mucus production	Fresh young hollow c.l. 5 luteal remnants of graded size	All phases followed in left ovary during clinical study	Clitoris somewhat enlarged	Some congestion.
8	B.Guernsey	1, 2.5 cm.	Came on heat weekly at one time. Considerable blood in tubal mucosa post-mortem	Cysts present throughout 35 days: also, slack cervix, copious oestrous mucus most of the time.	2 corpora lutea, slightly and very hollow, and fresh unovulated luteal body 1.25 cm Several very old remnants	Nil	Digging vigorously with fore feet	Set of empty fœtal membranes in each horn and severe endometritis!

The demonstration that approximately normal cyclic luteal development can co-exist with chronic follicular cyst production, complicates the process of assessment of results in treatment trials, past and future; and gives a warning against the danger of a hasty misdiagnosis of " retained corpus luteum ", etc. in the majority of such cases, without relaxed ligaments. These might benefit more from rupture of the large cyst in the other ovary, or still more from the management changes which are indicated most strongly in cystic disease. No solid support is forthcoming from these 13 cases for Hancock's (1949) view, that œstral mucus production and cervical opening are at their greatest in the presence of some form of normal genital phase succession, for mucus production and opening are much better evidenced (see below) in nymphomaniac cases without co-existent c.l. production. Although associating poor opening and cervical gland activity with " degrees of

luteal deficiency", it may be that Hancock did not have frank nymphomania in mind when he published this statement.

The Ovary in Simple Nymphomania

The second and larger sub-group, in which chronic active follicular cyst formation is unaccompanied by luteinisation, is now considered. For convenience, one may assume that when heat-like signs are shown, œstrogen production is taking place, and that production is at a low level in the absence of these signs. This assumption is based on Garm's observations on the appearance of birefringent crystals in the follicles, on Yumauchi's quantitative results on cyst œstrogen assay, and on the latter author's work, confirmed by Dubreuil (6), on the differing structure of the theca interna in "active" and "degenerate" cysts. The hypothesis of Hancock that œstrogen, present in full quantity in anœstrous cases, is inactive from shortage of priming progesterone, was put forward before publication of the above-mentioned results. The aspect of most interest in the analysis of these cases must be the extent to which œstrogen production is continuous, and the absolute magnitude of the production. For the present material the estimate was made by tabulating the exhibition of "heat-like signs", including the behaviour; the state of the pelvic ligaments, the degree of opening and the secretory state of the cervix, and also the vascularity of the tubal mucosæ. Cembrowicz (1946) has, *inter alia*, described the increased vascularity of the normal oviduct during œstrus; in the present study a gross degree of such vascularity occurred often enough to afford useful circumstantial corroborative evidence of œstrogen production. Data from 26 "simple nymphomaniacs" were analysed according to the method set out in Table III, which includes only four cases as examples. The volume of œstrous mucus—"slime"—produced was estimated arbitrarily; the continuity of its production is expressed as a fraction showing the proportion of serial clinical examinations at which it was found. No such detail of the cervical condition in this disease appears to have been recorded previously. The degree of opening of the cervix tabulated is the "average degree" of all the clinical inspections.

TABLE III

Bases of Oestrogen Production Rating

Case	Duration of Disease	Tail-head (pelvic-ligament)	Tubal Mucosa	Slime Production		Opening of Cervix	Behaviour	Oestrogen Production Rating
				Continuity	Volume			
1	16 days; after 14 mths cyclic infertility	Normal	Normal	3/4	Copious	Open	Anoestrous but erratic	Low
2	7 weeks; after 14 mths cyclic infertility	Somewhat raised	No Vascularity (severe damage)	3/4	Scanty/ moderate	Half open	Continuous oestrus	Moderate
3	8 months	Raised	Very highly vascular	4/5	Moderate	Wide open	Continuous oestrus	High
4	12 months	Normal	Normal	1/5	Moderate	2/3 open	Anoestrous	Low "Terminal"

In five cases œstrogen production was considered to be low; besides case 1 of Table III, in which the disease actually arose during the clinical study, there were two others with similar evidence of low œstrogen production and there was one other case besides case 4 with low production which was judged to be "terminally

low ". Both of these had at one stage undoubtedly been in high active œstrogen production. The " moderate " œstrogen producers formed the largest group with 10 cases, but the " high " and " very high " with four cases each were, together, of comparable size; three of the four very high cases showed some denaturation of the pelvic bone structure. No œstrogen estimate could be made in the remaining three cases because of lack of data. The average number of clinical examinations, positive for œstrous mucus production, in this group of 26 was 3.75. The average number of examinations made was 4.6, so that the percentage of examinations at which œstrous mucus was found reached 81.5. Volume of mucus was estimated as on average, copious in five, moderate in seven, and intermediate in four cases. The mean degree of cervical opening, on the arbitrary estimate worked out at 2/3 open. Superficially there appeared to be some correlation between the magnitude of the œstrogen production and the length of standing of the case. Two cases only developed during the clinical study; three showed short duration recurrences of the disease over long infertility periods; five ran courses of up to four months; seven, 4-8 months, and seven, 8-15 months. In 19 of these 26 cows an estimate of the condition of the pelvic ligaments was made; 13 showed relaxation. In six it was very marked, and in six the ligaments appeared normal. In 16 it was possible to assess the behaviour, seven showing continuous œstrus, two œstrus at short irregular intervals, one regular œstrus, and six were rated as " anœstrous ", despite quite good evidence of œstrogen production in three of them.

Some interest attaches to a corresponding analysis, including œstrogen production estimate, of the 13 cases with regular corpus luteum formation. In seven œstrogen production was considered to be low, probably terminally in one where it had formerly been high. The remaining six cases all indicated a moderate œstrogen production. The average number of clinical examinations positive for œstrous mucus production in this group was 4.1, and the average number of examinations made 8.5, giving the percentage of mucus-positive examinations at 48.5, a probably significant contrast with the figures of 81.5 per cent for the " straight " nymphomaniacs. The cervices averaged less than half " complete " opening. One case developed during the clinical study; the others ran long courses. Although six of the cases did show relaxed ligaments, the extent to which they were relaxed was on the whole less in this group. None showed continuous œstrous behaviour, two appearing anœstrous, three regular and five showing frequent irregular and sometimes prolonged heats. As many as seven of the " straight " cases showed high vascularity of the tubal mucosa; despite the accompaniment of severe cicatricial changes in the tubes of two animals. Similar pathological alterations may have masked this feature in another five cows. As experimental treatment produced response in a further four, and might, thus, have cleared this congestion, the percentage of cases showing high vascularity, amongst those in which it might reasonably be expected, reaches 40. Furthermore, it seems that the avascular tube cases (60 per cent) include several of those with the lowest œstrogen production as estimated on other criteria. One of the cyclic nymphomaniacs also exhibited highly vascular tubes.

The Ovary: General Considerations

The relative incidence of cysts as between the right and left ovaries was considered. Examination post-mortem was made on the 48 cases so far discussed (except for a few cows which were cured or improved by treatment). The examinations revealed 31 per cent with cysts in the right ovary, 28 per cent with cysts in the left ovary and 41 per cent with bilateral cysts. These figures are in fair agreement with those of Roberts (1955), from the eastern U.S.A. who found 33, 23 and 44 per cent respectively. They differ, even more widely than Roberts, from the Scandinavian results, which all show much lower figures for the left ovary, to the " advantage " of the right. This possibly reflects a breed difference. However, if the post-mortem results be considered in relation to the clinical studies on these cows, it is found that many unilateral cases at post-mortem were undoubtedly evenly bilateral during life, and correspondingly, two cases bilateral at post-mortem had been considered to be unilateral on clinical examination during life. Transferring the " clinically bilateral " cases from the unilateral figures gives a revised proportion of 22 per cent unilateral in the right ovary, 19 per cent in the left and 59 per cent bilateral; this latter is a higher figure than has been recorded by any previous writer. Most of these were, presumably or admittedly, working with single assessments per cow. The integration of clinical studies with post-mortem findings would seem to indicate that the superior activity of the right ovary is less marked than it is in a normal cow, and that the bilateral cystic condition is the one most typical of cystic disease. This result would probably follow from excessive stimulation of two organs, both of high, but of slightly differing responsiveness.

The mean diameter of the largest cysts found in each of the cases in this series was 2.86 cm.

Garm recorded the occurrence of islets of luteal tissue, also thin patches of luteal cyst lining, in the follicular cysts of a number of the true nyphomaniacs. Similar small masses of luteal tissue occurred in cysts from 14 of the 39 simple and cyclic nymphomaniacs in the present series. In four others the cystic fluid was of a bright orange colour instead of being water-clear. In a further three, small spheres of fresh luteal tissue were found buried deep in the ovary, apart from the cysts. They were from 4-8 mm. in diameter.

The Nymphomaniac's Uterus

On clinical and macroscopic examination, the finding of *enlargement* occurred only once in the 39 organs studied here. The enlargement was gross and associated with pyometra to the size of a three-months' pregnancy. One of the virilism case series showed a similar degree of enlargement. Garm in contrast, found enlargement of the uterus in 49 per cent of 62 nymphomaniacs studied. The difference in incidence is possibly more apparent than real, perhaps it may be a matter of terminology, since Garm attributed the enlargement to " œdema ", resulting from a diseased condition of the adrenal cortex. In the present series the average *tone* of the uterus during life was estimated as " markedly subnormal ", a consistent finding in 13 of the cases, or 33 per cent. The pyometra case was excluded. " Œdema " might normally be expected to affect muscular tone. If one assumes

that a similar condition was described as enlargement by Garm, agreement is reasonably close. The uterine tone was normal in 17 cows, and in six it was above normal. This rise might be attributed to the effect of the œstrogens in circulation for they are said to produce this effect in normal œstrus. It was curious that as many as three of the raised-tone cases occurred in the small group of 13 cyclic nymphomaniacs. This could be accounted for on Hancock's and Garm's assumption that the presence of a corpus luteum protected the adrenal cortex against the worst effects of continuous œstrogen stimulation. Conversely, in simple nymphomania, more advanced cortical disease might induce œdema by upsetting electrolyte balance (Garm), thus masking the normal tone-raising effect of œstrogen.

Histopathologically also, a difference was apparent between the 13 cyclic and 26 simple cases of nymphomania. Assessing "moderate" endometritis on the criterion that most glands of an endometrial section were filled with polymorphs and lymphocytes, with some general infiltration and fibrosis; and "severe" endometritis on the disappearance of all or most of the glands, with more extensive fibrosis, etc.; then in the cyclic series 20 per cent showed severe pathological change, and 25 per cent slight, while in the simple series 45 per cent showed severe endometritis, 23 per cent moderate and 6 per cent slight. Thus the incidence of endometritis was at least twice as high in the simple as in the cyclic group. The occurrence of cystic glandular dilatation bore no relation to that of inflammatory change; often it was apparent in severely fibrosed and invaded endometria, as the photographs show. It was seen also in otherwise normal ones. It is possible that some cases of dilatation were completely masked by the extent of the endometritis in the section. Only 40 per cent of the simple cases exhibited dilatation, and to a very varying degree. In the cyclic group 60 per cent showed dilatation, confirming that the higher incidence of severe endometritis may have masked further dilatation cases in the simple series. Garm found some degree of dilatation to be "usual" in his series.

From the bacteriological aspect, Garm himself took the view, that his study showed an atypically low incidence of invasion of the uterus by proliferating organisms of the normal vaginal flora, in a system flooded with œstrogen. He found only two cases of visible discharge, and recovered a few organisms in culture from six of 52 cases. The present writer in his review paper (1957) confirmed that these results were atypical, and adduced evidence of a fairly high incidence of important secondary bacterial activity from four authors, none of whom, however, carried out a detailed and balanced study. In the present series cultures were examined from 34 uteri, of which two yielded *Corynebacterium pyogenes*, two yielded streptococci, and two hæmolytic or non-hæmolytic staphylococci. In addition, a further two yielded moderate densities of staphylococcal colonies, and 10 yielded a few colonies of cocci, anthracoids, or *Bact. coli*. No organisms were obtained on culture from 16 uteri. Two of these latter, as well as two which yielded cocci only at post-mortem, were associated, during the period of clinical study, with the production of large quantities of pus yielding *C. pyogenes*. Thus 18 organs out of 34, or 53 per cent, yielded organisms, as compared with Garm's 11 per cent, and six organs yielded large numbers of possible pathogens, besides the three cases which yielded *C. pyogenes* abundantly in discharge during life, thought sterile (2), or with a

few non-pathogens (1) at autopsy—these exhibited endometria which were grossly damaged. In a total of nine cases or over 25 per cent the organisms were present in such a way that they probably complicated the disease. Garm's view that his material which did not yield such a picture was not typical is thus substantiated, and the high incidence of associated uterine infection, presumed and recorded by Romiti (1955), Gruber (1952), Hoeckner (1952) and Grandchamp (1953), is confirmed also, this time with some bacteriological and histopathological background.

TABLE IV

Infertile Cyclic Controls	Post-mortem Culture Results	Nymphomania Series	
2	Innumerable or Numerous C. pyogenes	2	
-	Innumerable / Numerous haemolytic or non-haemolytic cocci	4	
2	Total yields of possible pathogens in high density	6	Overall Total Possible Pathogens
2	Cases yielding Innumerable C. pyogenes during clinical studies only, and	3	
4	Not included above	9	

Table IV compares the incidence of uteri associated with possible pathogenic yields of organisms on culture, with that in 34 infertile cyclic controls (i.e. other types of barren cow), selected at random. This table too affords confirmation of Garm's view that the flooding of the system with œstrogen may well facilitate the invasion, and precipitate the pathogenicity, of organisms from the vaginal flora, according to the principle demonstrated in the mouse by Weinstein, Gardner and Allen (1937) and discussed by Romiti as well as in the present writer's earlier paper.

The high incidence of pathological change, as described, is, of course, consistent with the bacteriological results recorded above; further, the markedly lower incidence of endometrial pathology in the *cyclic* nymphomaniacs, may tentatively be ascribed to the bacteriostatic or even " antiseptic " effect of some kind of regular *change of phase* in the reproductive organs, as was suggested by this writer in connection with the treatment of endometritis in 1949 and 1951. Certainly the results now reported are no more consistent with the view, that œstrogens are in themselves disinfectant, than was the finding of eight cases of cystic ovarian disease, 28 with empty inactive ovaries, and only two with any form of ovarian cycle, in a group of 63 cases of purulent infective endometritis studied in 1944-8.

Conclusion

The most striking general feature indicated by the clinical-pathological study was the consistency of the findings suggesting a continuous high œstrogen production in the ovaries; and the clear-cut distinction between the cases in the cyclic and simple series with regard to the apparent œstrogen production rate and, as may be

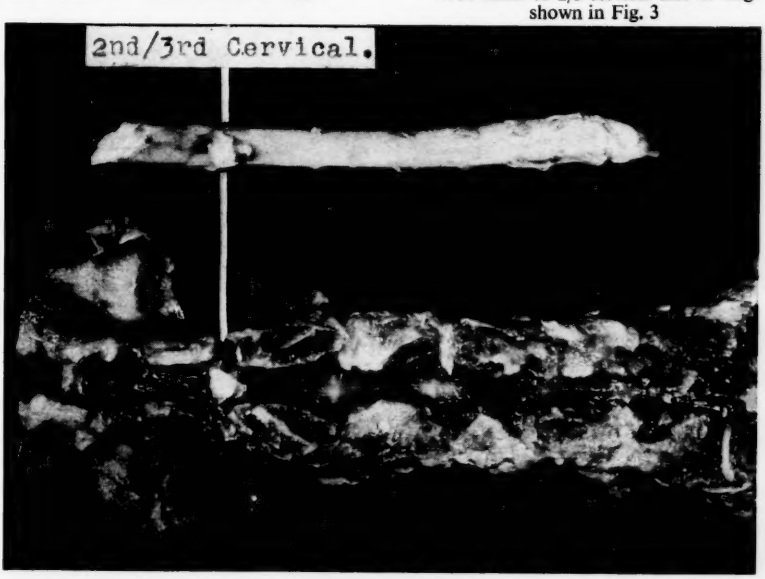
PLATE I

Fig. 1
5-year-old male spaniel exhibiting posture adopted with acute cervical pain.

Fig. 2
Protrusion of 2/3 cervical disc (a) in the dog shown in Fig. 1

Fig. 3
6-year-old female spaniel with quadriplegia, Demonstrating inability to stand.

Fig. 4
Protrusion of 2/3 cervical disc in dog shown in Fig. 3



(Article by L. C. Vaughan on page 105)

PLATE II

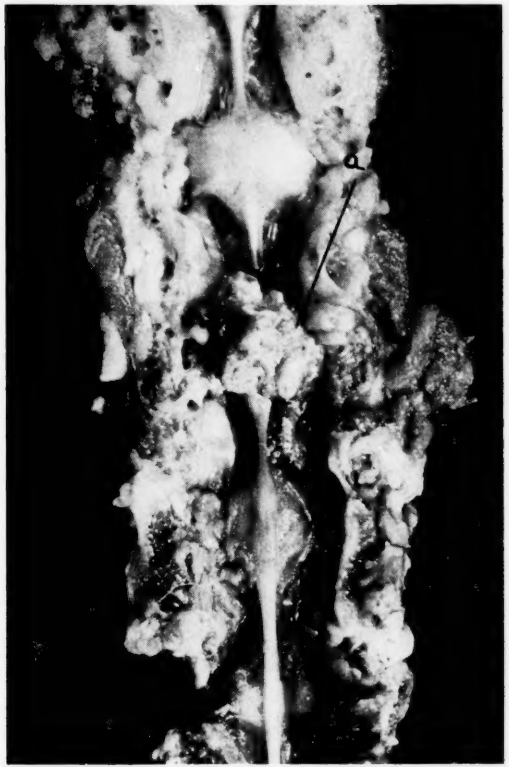


Fig. 5

Protrusion of 6/7 cervical disc (a) in a 6-year-old male pekingese.

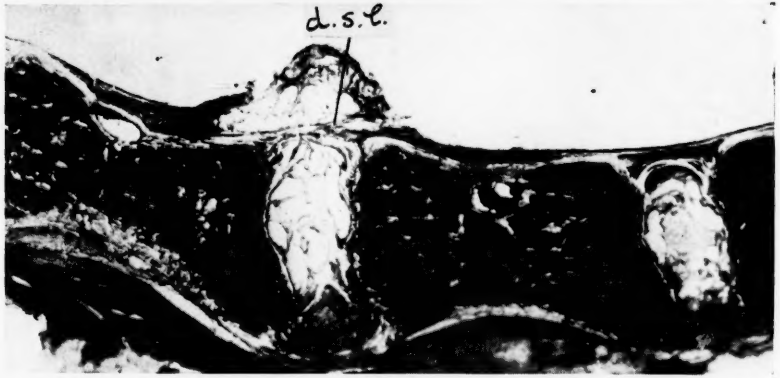


Fig. 6

Sagittal section of spine at level of 6/7 cervical disc (cut by Gough-Wentworth method) protrusion shown in Fig. 5. Note the intact dorsal spinal ligament (d.s.l.) in this section 1 m.m. from the midline

Fig. 7

As for Fig. 6. Section in the midline. Note ruptured dorsal spinal ligament (d.s.l.) and nucleus pulposus in communication with the protrusion

(Article by L. C. Vaughan on page 105)

PLATE I

Fig. 1

Endometrium, otherwise near normal, showing a gross degree of cystic glandular dilatation. (Above) × 70. (Below) × 440.

Fig. 2

In the gland under high power, the necrotic glandular epithelium has become detached from the upper-left hand margin.

Fig. 3

Severe endometritis, still accompanied by marked glandular dilatation (at the epithelial lumen surface). Note absence of luminal epithelium and, in left-hand bottom corner, 5 normal size gland sites now occupied only by fibrous tissue. (Similar gland sites occupy the *greater part of the area* of this section). × 70.

(*Article by F. L. M. Dawson on page 96*)

PLATE II

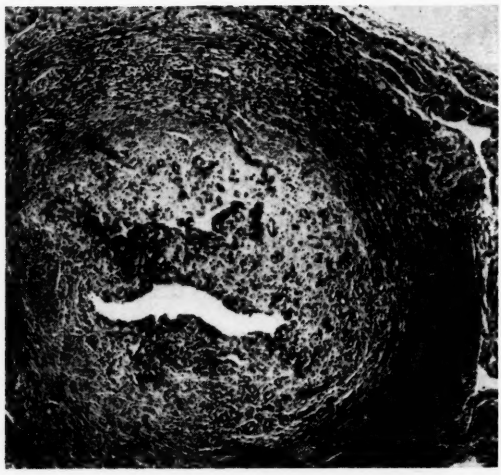


Fig. 4

Transverse section isthmus of oviduct from nymphomaniac with normal bursæ but bilateral endosalpingitis. No endothelium; fibrosis; diametrical septum formation.

(Judged impassable to ova.) × 70.

Fig. 5

"Severe endosalpingitis" in mid-section of nymphomaniac's oviduct. Function not necessarily destroyed. No endothelium; distension of mucous folds as by inflammatory exudate pressure. Some fibrosis and cell infiltration. × 35.

Fig. 6

Destruction of function inside oviduct ampulla, associated with bursitis. Multiple septum formation and other changes. Photograph includes most of the transverse section

(*Article by F. L. M. Dawson on page 96*)

inferred, the serious effects of the " higher " rate obtaining in the simple form of the disease, upon the uterine, and tubal (see below) tissue, and presumably on prognosis. The occurrence of a by no means negligible incidence of heat-like signs, in half the cases whose *behaviour* was rated as " anœstrous ", suggests, that in the past too much importance may have been attached to behaviour observations not correlated with clinical studies. It may be that the possibility of cystic disease, apart from œstrogen production, has at times been regarded unduly seriously. An excellent example is furnished by the protagonists of the origin-through-ascending-infection school, which flourished in the 1920's and is not yet extinct. In the present study even the incidence of " terminal " low œstrogen cases was found to be rather surprisingly low, and the impression was formed that œstrogen production had a tendency to rise steadily and indefinitely, and in the absence of treatment for the condition to become aggravated steadily with the lapse of time. The correlation between the occurrence of denaturation of pelvic bone, and other signs suggestive of the highest œstrogen production, was so close as to carry some conviction although only four animals were involved. Garm did not encounter bone lesions in his series of cases.

The condition termed, in the review " intermediacy " was encountered, most frequently in the cyclic cases, but the cases are so few that the observation can be only of doubtful significance. Five cases in the whole series of 39 nymphomaniacs exhibited bull-like *behaviour* including digging with their horns and fore feet. Only three of these were definitely interested in bulling cows and were more willing to " jump " than to " stand ". A sixth cow had a very masculine *voice* but she was not of masculine appearance or behaviour. Careful routine examination of the clitoris region began only after a number of the subjects had been killed. Two cases, however, had enlarged clitorides but there were no other signs of masculinisation. The percentage of cases showing intermediacy was about 20, as contrasted with the 40 mentioned by Vandeplassche. This worker lays stress on the evidence of the clitoris, so the figure of 20 for the British series can be regarded as a minimum one only.

STUDIES ON INTERVERTEBRAL DISC PROTRUSION IN THE DOG*

By L. C. VAUGHAN

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1. ÆTIOLOGY AND PATHOGENESIS

Introduction

PARAPLEGIA in the dog, with its many causes, is an ever present problem in veterinary practice. Paraplegia may be the result of spinal injury and quite frequently it is a sequel to infection with neurotropic virus. Dachshund and Pekingese dogs in particular are prone to a paraplegia which had the following characteristic features. As a rule the onset of paralysis is sudden, there was no history of previous

* This work was included in a thesis for Fellowship of the R.C.V.S.

trauma or infectious disease and it is not unusual for paralysis to recur in cases which have partially or completely recovered from a previous attack. Such is the breed incidence that frequently the term " Dachshund paralysis " is applied. Dogs of the same breeds are afflicted occasionally with acute myalgia to which the terms " rheumatism " and " lumbago " are given.

Present knowledge would suggest that the conditions mentioned above are due primarily to rupture of the annulus fibrosus of the intervertebral disc and pressure on the spinal cord by the extruded nucleus pulposus. The author, whilst acknowledging the assistance received from the review of foreign literature, is conscious of the dearth of information on the subject of disc protrusion in this country, and as a result of his own investigations has been able to show that in canine orthopædics herniation of intervertebral disc substance plays an important role in the production of signs and symptoms, varying from cervical and lumbar pain to paraplegia or even quadriplegia (Figs. 1, 2, 3, 4).

Literature

Dexler (1896) was the first to describe a form of paraplegia in the dog which was due to compression of the spinal cord by cartilaginous excrescences of the intervertebral discs. He called the condition Enchondrosis Intervertebralis but there is little doubt that at the present time it would be described as an intervertebral disc protrusion. Dexler's term for the condition was used by Hutyra, Marek and Manniger (1922) and Gaiger and Davies (1932). Calcified intervertebral discs, demonstrated radiographically in dogs showing symptoms of pain or paralysis, has been held to be the cause of these syndromes (Schnelle (1938, 1945), Kirk (1939, 1942) and Holmes (1942)). The first worker, however, to suggest that the lesions typical of Enchondrosis Intervertebralis were protruded intervertebral dics material was Tillmans (1939). Support for this view later came from Love (1939), Schlotthauer (1940) and Riser (1946). During the last decade studies on disc protrusion in the dog have taken place in Germany (Fankhauser, 1948), America (McGrath, 1951; Hoerlein, 1953) and Sweden (Olsson, 1950, 1951a, 1951b; Hansen, 1951, 1952; Olsson and Hansen, 1952; Hansen and Olsson, 1953). In Great Britain little has been published on the subject. Boddie (1949) asserted that disc protrusion, in a manner comparable to that seen in man, was anatomically impossible in the dog. The present investigation shows that this statement is not correct. Smith and King (1954) reviewed the literature on disc protrusion in the dog and recorded some clinical observations of their own and later (King and Smith, 1955) gave an account of some comparative anatomical studies which they had made on the spines of man and dog. Myelography in the diagnosis of disc protrusion has been discussed by Douglas (1955).

Materials and Methods

The material which forms the subject matter of this investigation was obtained at the Field Station of the Royal Veterinary College at Streatley. The majority of cases exhibiting symptoms which might be ascribed to disc protrusion passed through my hands. Between May 1952 and July 1956, 65 dogs with intervertebral disc protrusion were investigated and an attempt has been made to correlate the pathological lesions with the different clinical manifestations of the disease.

The routine procedure adopted included clinical examination of the animal on admission to hospital, where the majority were kept for observation, followed by radiographic examination of the entire vertebral column. Myelography after intrathecal injection of a contrast medium was undertaken in all instances where it was essential to diagnosis and unlikely to jeopardise the animal's chances of recovery. Therapeutic measures were adopted according to the signs exhibited, the severity, site and age of the protrusion. Surgical intervention was instituted in 16 dogs when conservative measures had registered little response. Disc fenestration was performed on 13 and laminectomy on 3 dogs. Dogs which died or were destroyed were subjected to a thorough examination post-mortem. The vertebral column was dissected and examined in detail and when possible a histological examination of the spinal cord and protruded disc material was made.

Ætiology

It is recognised that the intervertebral disc undergoes degenerative changes with age to a degree not found in any other body tissue. Information regarding the changes that occur in the ageing canine disc has been contributed by Hansen (1952). In the so-called chondrodystrophoid breeds (Dachshund, Pekingese, French Bulldog, Spaniel) the nucleus pulposus begins to lose its mucoid nature during the first year of life and, with few exceptions, by the time the animal is four years old, the nucleus has become cartilaginous and often it becomes calcified eventually. The annulus fibrosus undergoes structural alteration also. The lamellæ loosen and disintegrate or become hyalinised. By contrast, in the other breeds the nucleus pulposus becomes fibrous gradually as a result of collagenisation but it is only after the age of seven years that this process is advanced to significant proportions. At this time there may be changes also in the annulus similar to those described above.

As the disc loses its original structure so its ability to function as a shock absorber decreases and it becomes increasingly liable to injury. This is marked most in the chondrodystrophoid breeds in which the degenerative changes are advanced by early middle-age, a time when the dog is still capable of vigorous activity. The present work has confirmed the previous view that disc protrusion occurs most commonly in the middle-aged dog belonging to certain breeds (Dachshund, Pekingese, Spaniel) and Hansen's findings offer a reasonable explanation for both the breed incidence and the age susceptibility.

Medical opinion is almost unanimous in the belief that in man protrusion of the degenerating disc is frequently precipitated by some form of injury, associated with great physical stress, rather than direct trauma. In the dog, on the other hand, it is unusual to obtain any evidence of injury immediately preceding the onset of the trouble although in most cases it is not possible to prove that an injury or accident has not occurred. The repetitive mild trauma which is accepted as part of the dog's everyday activity may be an important factor and the final injury which causes the disc to protrude may pass unnoticed.

The possibility that some inherited factor exists which may predispose to disc protrusion should not be overlooked. Information regarding familial tendency is difficult to obtain because, as a rule, by the time the dog suffers from disc pro-

trusion its litter mates have become widely dispersed. In this study two families of Dachshunds were affected. In the first instance a mother and son were involved. In the second there were four related dogs of three generations. Their relationship was granddam (and her half-sister), her son, and his daughter. Little inference can be drawn from these facts but they do suggest a possible line of research.

During the present investigation a tendency for disc protrusion to occur in bitches while they were in œstrus or in the early pseudo-pregnancy period was observed. 10 of the 35 females examined became affected at this time and it seems possible that this time relationship is significant. In the human subject with disc protrusion, O'Connell (1951) found that 17 per cent of his women patients became affected when they were pregnant or in labour. He postulated that the corpus luteum hormone might cause relaxation of the spinal as well as the pelvic ligaments and thus contribute to disc protrusion. In trying to find some correspondence between the present finding in the bitch and those of O'Connell in women, it may be pointed out that the hormonal relationships of œstrus are very similar to those of parturition while pseudo-pregnancy and pregnancy bear a striking similarity. From veterinary experience, however, and from the writer's observations, if there is a connection between disc protrusion and hormone balance, the œstrogenic rather than the progestational hormones are more likely to be involved.

Before leaving endocrine considerations it may not be out of place to reflect on the effect of gonadal and adrenal steroids on connective tissue. For example, it has been established that in certain connective tissue sites reversible mucoid—collagenous changes may be mediated by the sex hormones (Maximow and Bloom, 1942). The importance of a premature collagenous change in the mucoid tissue of the nucleus pulposus has been discussed already and it may well be that there is an endocrine background to this phenomenon.

In the dog the commonest route taken by the nucleus polposus in the formation of a protrusion is via the dorsal annulus and into the neural canal. The chief reason for this may be deduced from the fact that the nucleus is so eccentrically placed in the disc that the annulus is thinnest, and hence weakest, dorsally. In the present work dorsal protrusions were most frequently dorso-medial in position, and since the dorsal annulus is thinnest at this point this observation supports the foregoing view. The present writer agrees with Hansen (1952) and Hoerlein (1953) that disc protrusions in the dog most commonly occur at or near to the thoraca-lumbar junction. The relative magnitude of the mechanical forces and stresses in the different spinal regions in the dog have not been established. However, it might reasonably be expected that by virtue of its position in the vertebral column, the region of the thoraco-lumbar junction does bear considerably more strain than other spinal regions. The cervical region, where protrusions not uncommonly occur, is subject to additional stress and strain also.

It has been suggested that in man (Bradford and Spurling, 1947) the mobility of a spinal region, in association with disc thickness may be factors responsible for protrusion, since in the lumbar region, where mobility is great and the discs are thick, protrusions are most common. These factors are not of apparent importance in the dog since King and Smith (1955) have found that in the lumbar region (where

protrusions are most common) mobility is minimal and the discs are not as thick as those in the cervical region.

It has been observed that protrusions rarely occur at the nine discs between the first and tenth thoracic vertebræ, which is in support of the findings of Hansen (1952) and Hoerlein (1953). These workers suggest that the reason for this is the additional support given in this region by the conjugal ligament.

Pathogenesis

A characteristic feature of disc protrusion in the dog is the variety of symptoms which may be shown. It is obvious that signs and symptoms are partly determined by the situation of the protrusion since the clinical appearance of a dog with a cervical lesion differs markedly from one with a protrusion in the thoraco-lumbar region. According to Riser (1946) the degree of compression of the spinal cord may determine the type of the signs exhibited, e.g. pain, ataxia, paralysis. In the author's experience this is not strictly true. For instance, it is not unusual for a dog to show acute symptoms and yet at autopsy the lesion may be small and incapable of severe cord compression. Conversely, a lesion which from its size might be expected to have produced complete limb dysfunction has in fact caused pain or only mild limb disuse. Olsson (1951a) considers that in the determination of the signs the rate of development of the protrusion is more important than its size. He believes that this " dynamic factor " in some dogs is responsible for an initial acute paraplegia which is soon followed by a fatal ascending paralysis. In these cases usually there is evidence of an acute inflammatory reaction, with extensive hæmorrhage and a wide dispersal of the nuclear material, but with little compression of the cord. While the author is familiar with these clinical and post-mortem findings he doubts Olsson's theory, which pre-supposes the explosive emptying of the nucleus into the spinal canal. For it has been shown that by the time a protrusion is likely to occur the nucleus has undergone changes which have greatly reduced its fluidity (Hansen, 1952). In the present study with the aid of the Gough-Wentworth (1947) method, serial sagittal sections ($\frac{1}{2}$ m.m. thick) of the spine have been cut and we have been able to take measurements of the rupture site (Figs. 5, 6, 7). In six dogs it was found that the diameter varied from 0.5 to 1.5 m.m. It is difficult to visualise the explosive emptying of a chondroid nucleus through such a minute opening even when the pressure inside the disc is taken into account.

According to Hoerlein (1953) the important factor, apart from compression, in symptom variation, is the " pathological response " to the protrusion since " no two dogs will react similarly to a given protrusion ". There is no doubt that a protrusion provokes an inflammatory reaction, the severity of which may vary in different dogs, and this may be an important factor in determining the symptoms. In one dog with paraplegia, in the present investigation, an early myelograph revealed an obstruction in the subarachnoid space 4 vertebræ anterior to the true site of the compression. The latter was discovered a week later. Presumably the high level of obstruction in the first instance was the result of the inflammatory reaction to the protrusion, for at autopsy the latter was comparatively shallow.

Since the spinal cord in the dog almost completely occupies the neural canal, the effect of compression is borne by the entire cord at that site. This, together

with the fact that the protrusion is usually dorso-medial in position, explains why limb symptoms are mostly bilateral. Only occasionally are there unilateral limb symptoms and in these cases the lesion is either dorso-lateral in position or the protruded mass has deviated more to one side of the midline. Pain is most likely due to the irritation of the cord and/or spinal nerve roots. The pain in some cases appears to be widespread and this is explained by the fact that a spinal nerve supplies sensation to muscles, bones, joints and ligaments far beyond the range of its cutaneous supply. For the same reason it can be appreciated why the dog cries with pain at every movement.

Recovery from disc protrusion is dependent in the first instance on the extent to which the cord has been injured, either by compression or interference with its blood supply. It is impossible to assess the true extent of the injury and so the prognosis, especially in the case of a dog exhibiting severe symptoms, is always in doubt. In the present work radiographs were taken of a number of dogs 3-4 weeks after myelography and in several it was found that globules of Lipiodol had descended beyond the obstruction. This finding suggests that a decrease in the size of the protrusion and/or the inflammatory reaction occurs frequently. These observations are not in agreement with Olsson's findings that the subarachnoid space always remains obstructed to radiopaque material even in those dogs which recover. Hansen (1952) has shown that a protrusion may diminish by resorption. This may explain the reduction in size of one calcified protrusion in Olsson's series and of 3 such protrusions in the present work. Many protrusions remain permanently, even in dogs which have recovered, and it is remarkable how well the cord becomes adapted to compression.

A relapse after a partial or complete recovery has been made is another feature of disc protrusion. This may be due to the occurrence of a new protrusion or to an increase in size of the existing lesion. The symptoms shown at relapse do not necessarily simulate those shown originally. In most instances the new lesion occurs in close proximity to the first. It may, however, occur in a different region of the spine. Relapse occurred in 6 dogs in the present series and the intervening period between the attacks varied from one month to 2½ years.

Discussion

It is accepted by most observers that intervertebral disc protrusion occurs most frequently in the middle aged (5-6 years) dog of the chondrodystrophoid breeds (Dachshund, Pekingese, Spaniel). It is a point of major significance that by middle age the discs in these susceptible animals have undergone advanced degenerative changes. The cause of this degenerative process is not known. The author suggests here that hormonal and hereditary influences may be important and that future research may be directed along these lines with profit.

The degenerate disc is unable to withstand the trauma encountered in the dog's every-day life and consequently the annulus fibrosus and dorsal spinal ligament rupture and so allow the nucleus pulposus material to be extruded. That the route of escape from the disc is dorsal can be explained because the annulus is thinner and hence weaker in that area. Although the degree of degeneration is uniform throughout the spine, the discs most liable to protrusion are those situated at or

near the thoracolumbar junction and in the cervical region. The majority of protrusions occur in those spinal regions which bear the major stresses and strains. This gives support to the contention that trauma is a major factor in the production of the protrusion.

The signs which may be exhibited as the consequence of a disc protrusion vary tremendously. It is obvious that the signs produced by the protrusion of a cervical disc will differ from those produced by a protrusion in the thoraco lumbar region. Formerly it was thought that, apart from the regional location of the protrusion, variation in signs and symptoms was dependent on the degree of compression of the spinal cord. Whilst this is often the correct explanation, experience has shown that it is not always the case. It is not unusual to find at the autopsy on a dog which had shown acute signs and symptoms that the protrusion is small and incapable of severely compressing the cord; the converse may be found. Olsson (1951) believes that the rate of development of the protrusion is the important factor and he visualises that the nucleus pulposus material may be propelled with considerable force against the spinal cord, in a dog exhibiting acute signs and symptoms. The author has found that, even allowing for some eventual contraction, the rupture is minute and it is difficult to imagine the explosive emptying of nuclear material (which, at this time of the dog's life, is chondroid in nature) through such a small aperture. Myelographical studies have shown that the degree and extent of the inflammatory reaction caused by the protrusion varies and it may be that the variation in signs and symptoms is dependent upon this factor.

In future communications other aspects of intervertebral disc protrusion in the dog will be discussed. These will include the diagnosis, the pathological findings, found on examination post-mortem of 32 dogs with disc protrusion; and the medicinal and surgical measures employed in the treatment of the various forms of the lesion.

I wish to record my thanks to Professor C. Formston, and to my colleagues in the department of surgery at Streatley and to Mr. J. W. H. Holmes for their help and encouragement and for all the facilities and materials which they have provided.

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CANINE VIRUS HEPATITIS IN SUCKLING PUPPIES

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Introduction

SINCE Rubarth's classical studies (1947) interest in canine virus hepatitis has been concerned mainly with the disease in adult dogs and the pathogenicity of the virus in suckling puppies has not received much attention. It was thought advisable to conduct an experimental study of the infection in suckling puppies and in particular to determine their susceptibility and the types of pathological lesions produced. The results of this study are presented and discussed.

Materials and Methods

1. *Experimental Puppies*

Suckling puppies were of different ages and breeds. Normal developed puppies of 60 days gestation were used, a total of 83 suckling puppies being inoculated with the virus. It was proposed to study the change of susceptibility with age, the significance of falls in post-natal body temperature and the role of milk of the dam in innate resistance.

2. *Virus Inoculum*

The virus material was liver obtained, for each set of experimental inoculation, from a fatal case of the disease. The tissue was ground up finely and suspended in saline to give a final concentration of 1 : 10. The suspension was centrifuged at 3,500-4,500 r.p.m. for 20 minutes and the supernatant fluid with 50 units of penicillin and 50μg. streptomycin per ml. was used as inoculum.

3. *Clinical and Pathological Examinations*

The clinical, post-mortem and histopathological findings in the suckling puppies were compared with those observed in dogs after weaning.

4. *Serological Tests*

Specimens were examined by the complement fixation test described previously (Larin, 1951a and b; Larin and Orbell, 1956).

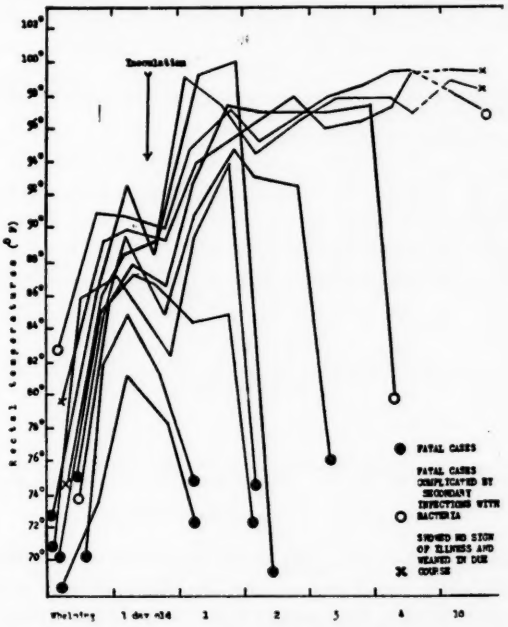
Wherever other techniques or materials were used they are described under corresponding headings.

Experimental Infection

The mortality rate produced by intraperitoneal inoculations of the virus into suckling puppies varied considerably from litter to litter. In some litters the puppies remained normal in the others age and extensive falls in the post-natal temperature probably influenced the results of the virus inoculations. A typical example of the effect of temperature falls during the immediate post-natal period is given in Fig. 1.

FIG. 1

The response of ten one-day-old suckling puppies to intraperitoneal inoculations of canine hepatitis virus.



The experiment shown in Fig. 1 was conducted during warm weather in July. Temperatures in the kennel maintained a steady level from 75-80° F. The litter of 10 greyhound puppies was born of a serologically-negative bitch in little over two hours and on the sixty-eighth day. The puppies were removed at birth and placed in a box containing a hot water bottle and blanket. The puppies were of normal size and none had any congenital malformation. Some immediate fall of temperature occurred in all the puppies. The individual temperature patterns are shown graphically in Fig. 1. The average of the rectal temperatures taken immediately after whelping was 74.9° F. or a decline of about 26°-27° F. from what must have been the original temperature when the puppies were born. In some

puppies the fall in temperature was as great as 32°-33° F. All ten puppies were inoculated intraperitoneally with active virus on the first day after whelping. Five of the 10 inoculated puppies, whose temperatures taken immediately after whelping ranged from 73° to 68° F., died from uncomplicated virus hepatitis and within three days of inoculation. Of the other five puppies, whose temperatures after whelping ranged from 82.9° to 73.9° F., two survived and three died. Of the latter three, one puppy died on the third day from uncomplicated virus hepatitis and the other two died of the disease on the fourth and tenth day respectively. The latter two cases were complicated by streptococcal broncho-pneumonia.

All the puppies were in good condition when the virus was given and death was sudden in almost all cases. In a very few cases signs of illness were observed from a few hours to a day or so before death. The affected puppy was less active and not sucking well. In some cases the mucous membrances of the mouth were slightly cyanosed.

FIG. 2

The Response of two 4 weeks old weaned puppies to intraperitoneal inoculations of canine hepatitis virus

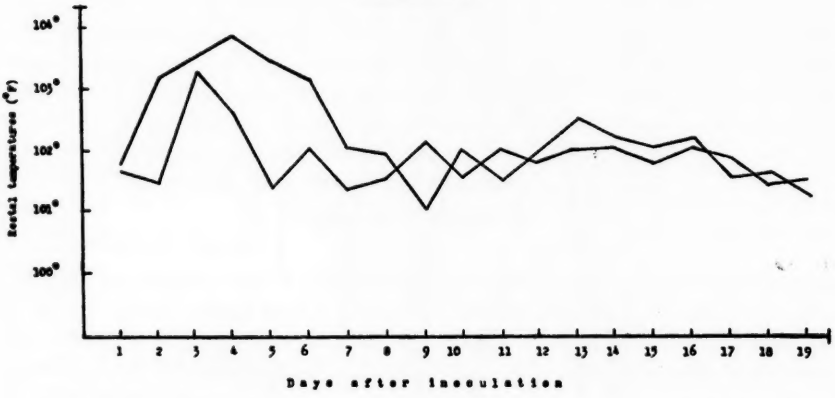


Fig. 1 shows that after some fall in body temperature during the immediate post-natal period a rising trend sets in. The temperature may go up to 98-100° F. in about 48 hours. During this period individual temperatures fluctuate widely and Fig. 1 shows the difficulties in determining whether the puppies developed a febrile reaction after virus inoculations. Fig. 1 shows that two puppies whose rectal temperatures fell to 75° F. and 79.9° F. during the immediate post-natal period, survived and were weaned normally. No complement fixing antibody was detected in the blood of these puppies on weaning and two months later. The blood of the dam was serologically negative at weaning. These two puppies were inoculated intraperitoneally with canine hepatitis virus two months after weaning. From the third day after inoculation onwards, the puppies developed fever of 103.5° F. to 104.6° F., tachycardia and reduced food intake; occasionally they showed blood-stained stools. After five days of malaise the puppies showed complete clinical

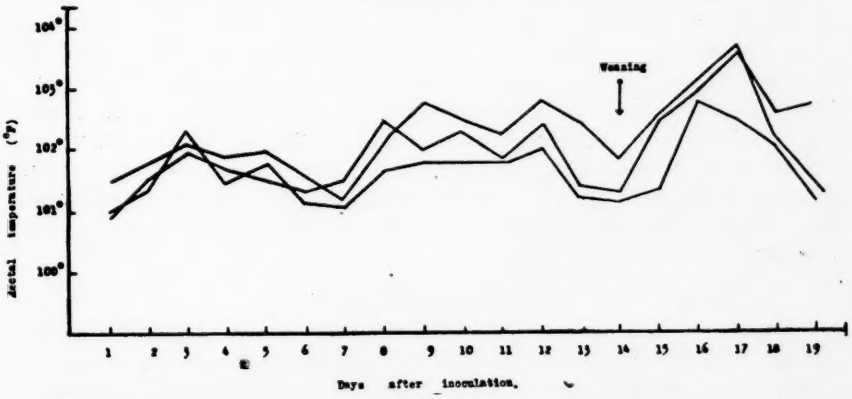
recovery and two weeks later they showed a good titre of the complement fixing antibody in their blood.

From these experiments it follows that (*a*) one-day old puppies are susceptible to canine hepatitis virus, (*b*) puppies showing a considerable fall in the post-natal temperature (down to 74° F. and below) are probably less resistant to the virus, (*c*) the disease in young suckling puppies is of short duration and finally that (*d*) some suckling puppies (two in this experiment) may not be affected after intraperitoneal inoculation with the virus, and may remain non-immune to challenge inoculation with the virus after weaning.

In further experiments with suckling puppies it was found that although, in general, older suckling puppies were more resistant to virus inoculations, it seemed that in some cases the milk of the dam also played a part in resistance. Since this was thought to be of importance, further experiments were conducted to assess the significance of any inter-relationship that may exist between the innate resistance to the virus of suckling puppies and milk of the dam. A typical protocol describing an experiment conducted on a litter of five suckling puppies four weeks old is shown in Figs. 2 and 3, the puppies and the bitch giving negative reactions in the complement fixation test. Two of these puppies were weaned and fed on cow's milk, the other three puppies remained on the bitch. All five puppies were inoculated intraperitoneally with canine hepatitis virus. It is shown in Fig. 2 that on the third day after inoculation the two weaned puppies developed fever with temperatures up to 103.3°-104° F. There was also tachycardia, reduced food intake and blood-stained stools. Two weeks after convalescence both puppies showed a good titre of antibody in the blood.

FIG. 3

The response of three 4 weeks old suckling puppies to intraperitoneal inoculations of canine hepatitis virus.



The other three puppies which were left on the bitch, showed no reaction to the virus and no antibody in the blood within two weeks after virus inoculation (Fig. 3). By the end of two weeks the puppies were weaned and on the third day after the weaning they developed fever with temperatures up to 103.4°-104.1° F.

(Fig. 3). There was reduced food intake and blood-stained stools. All the three puppies recovered clinically and two weeks after convalescence there was a good titre of complement fixing antibody in the blood.

It follows that the infection in suckling puppies can be influenced by at least three factors such as the age in which infection occurs, the degree of a fall in the post-natal body temperature and milk of the bitch.

Naturally Acquired Infection

The experiment showed that, as with the disease in adult dogs, there are no specific clinical signs which can be considered characteristic of virus hepatitis in suckling puppies. The puppies in question usually die ("fade") within the first few days of birth and the question of malnutrition does not arise. As in the case in adult dogs clinical jaundice occurs very seldom in suckling puppies. Investigations of the disease in the new-born puppy are also impeded by the following, (*a*) none of possible congenital disorders can be excluded with confidence, (*b*) the intranuclear inclusion bodies and the complement fixing antigen which are considered to be characteristic of the disease in the fatal form in adult dogs, are undetectable in the great majority of suckling puppies (Larin, 1953; Larin and Orbell, 1956), and (*c*) no susceptible test-host, other than the puppy, is available for detecting the virus (Larin, 1957).

Post-Mortem Findings

Bodily conditions were good except in a very few older puppies which had been ailing before death. The most striking post-mortem appearance in most cases was generally congestion of organs and tissues. In very few subjects a small amount of blood-stained effusion was present in the peritoneal cavity. Petechial hæmorrhages and extravasation of blood in the alimentary tract were infrequent. Some cases showed hæmorrhagic infiltration of the subcutaneous tissues; in most the heart was dilated and flabby. The coronary veins and their branches were congested and stood out prominently. The ventricles of the heart appeared to be normal and there was no evidence of endocarditis. The large veins were distended with blood. This probably accounts for intense terminal passive congestion of the internal organs. The greatly increased amount of blood in the liver in some caused the capsula to be stretched tightly and the organ presented a characteristic dark red glistening appearance. In some animals the lungs were congested and œdematous but in others they were almost normal; the spleen and kidneys were usually congested.

Histopathological Findings

Specimens for histopathological examinations were fixed in 0.85 per cent aqueous NaCl (9 vols.) with 40 per cent aqueous formaldehyde (1 vol.), or alternatively in Susa or Zenker fixatives. The tissues were processed and embedded in paraffin. Sections of all the tissues were stained by Erlich's hæmatoxylin and eosin, Best's carmine stain for glycogen and Gordon-Sweet's reticulin impregnation method. Other techniques used for selective cases were Forsgren's stain for bile acids, Stein's test for bile pigments and the per-iodic acid-Schiff's reagent method

(P.A.S.). Occasional use was made of collagen stains and also of stains for fat and lipids.

Tissue sections were studied from puppies which had died from experimental canine virus hepatitis, and also from puppies who had died from suspected naturally acquired virus hepatitis. The latter puppies showed similar post-mortem and histopathological lesions to those in puppies experimentally inoculated with the virus. The extent and distribution of the lesions varied considerably in both groups of puppies from case to case, and even from section to section in any one case. Histopathological lesions found in the organs and especially in the liver of puppies dying within two or three days of birth were, in some cases, of that degree of development which made it certain that they must have been present before birth. Thus the possibility of intra-uterine infection must be considered. The histopathological findings which are presented in Table I show important differences between the liver lesions in 50 suckling puppies and in 50 adult dogs. All died from the disease in acute form, and to our best knowledge it was not complicated with another virus or with bacteria.

COMPARATIVE HISTOPATHOLOGICAL FEATURES OF LIVER LESIONS IN FATAL CASES OF CANINE VIRUS HEPATITIS IN SUCKLING PUPPIES AND ADULT DOGS

Lesions	*Suckling puppies* (per cent)	*Adult dogs* (per cent)
Parenchymal changes		
Swelling of cells	94	36
Central loss of cells	12	72
Peripheral loss	0	28
Eosinophilic necrosis	42	10
Pigmented cells	28	42
Hyaline in cells	28	14
Fat	48	2
Intranuclear inclusion bodies ...	0	100
Interstitial changes		
Heavy infiltration	0	14
Light infiltration	100	86
Kupffer-cell stimulation ...	24	36
Bile duct proliferation	5	28
Fibrosis	5	14
Distortion of reticulin	60	64
Hyaline around veins and in portal tracts	82	22

Discussion

Canine hepatitis caused by virus infection appears to be widespread (Larin, 1953 and 1957). Thus from the immunological point of view the new-born puppy must be considered with regard to phenomena resulting from the relationship of the virus to the dam. The specific histopathological lesions which have been found in the occasional puppies which die from the disease soon after birth suggests that an

inter-uterine infection could have been responsible and that the virus may thus be able to cross the placental barrier.

On the other hand, the present studies show that intraperitoneal inoculations of active virus to presumably susceptible suckling puppies born of serologically negative dams were not followed by severe illness and heath invariably. The main interest of these findings lies in the demonstration of marked variations of the suckling puppy's susceptibility associated with the age, milk of the dam and the degree of falls in the post-natal temperature. Similar variations of the susceptibility were observed also in suckling puppies under natural conditions—i.e. housed in kennels in which virus hepatitis was endemic.

The general impression, derived from this study, is that older suckling puppies are more resistant to the virus. It would seem likely that in the suckling puppy there may be fundamental differences between the immunological response of the neonatal period as compared with the later life. These points are of some significance not only with regard to the natural defensive powers of the new-born puppy against infection in general, but also as they may affect the selection of the best time for immunizing procedures.

The experiments yielded evidence suggesting that the milk of the dam may play a part in the resistance of the suckling puppy to the virus. The nature of this milk factor is obscure and although one is tempted to explain it by the activities of virus neutralising antibody, the presence of this in the dam's milk has not been proved. On the other hand, electrophoretic studies of canine sera which have been conducted at these laboratories, show that the concentration of globulins and particularly of gamma globulin in the puppy's serum at birth is less than in the dam's. Although gamma globulin appears in the puppy's blood soon after suckling, the time and concentration vary considerably from pup to pup in the same litter. This may be due either to variations in the puppy's ability to absorb gamma globulin from the dam's milk, or rather to some individual difference of age-conditioned protein—synthesizing powers, and of proteins in the dam's milk as limiting factors in neonatal immunology. Since the concentrations of gamma globulin which appear in the blood of new-born puppies after first suckling are usually greater than amounts of gamma globulin in ingested milk, it is possible that milk proteins stimulate synthesis of the immunologically important proteins in the new-born puppy and also forces governing resistance to infection. The importance of innate resistance to infections with canine hepatitis virus noted elsewhere (Larin, 1957).

The relationship of the new-born puppy's production of heat (and thus of the body's metabolism) to his innate resistance to the virus is of interest. Puppies are almost poikilothermic at birth and such small degree of temperature control that they possess cannot be expected to behave in a reliable fashion. It is thus not so much the instability of the new-born puppy's temperature that matters, as the degree of instability which could be considered normal, and the possibility that variations beyond that degree might become a menace to the puppy's survival in an infection. Although puppies can recover satisfactorily after extensive falls in the post-natal temperature a characteristic example given in Fig. 1 (and it must be recalled here that this particular experiment was conducted during warm weather in July) shows

that a decline in the post-natal temperature to 74° F. and below this figure considerably affected the innate resistance of the puppies.

Finally, there would seem to be no doubt that tissue lesions and especially liver lesions in suckling puppies dying from experimental or natural infection with the virus differ considerably from those produced by the same virus in dogs after weaning. This fact complicates post-mortem and histopathological diagnosis of the infection in suckling puppies. In the majority of cases post-mortem and histopathological diagnoses of canine virus hepatitis in suckling puppies can never be definite, for the examinations can only reveal some lesions which *may* have been caused by the virus. Thus anamnestic investigations are of paramount importance for diagnosis of the disease in suckling puppies.

It is not known why histopathological lesions produced by the virus in suckling puppies should differ from the lesions found in puppies after weaning, and why the inclusion bodies are undetectable in the majority of suckling puppies. It is possible that the answer to these questions may be found by studying certain aspects presented by the new-born organism in his reactions to infectious agents. On the other hand, the relationship of the intranuclear inclusion bodies to canine hepatitis virus is supported only by the fact that numerous inclusion bodies may be found in lesions at histopathological examination of fatal cases of the disease in dogs after weaning.

Summary

Experimental data shows that new-born puppies are susceptible to canine hepatitis virus. The infection in suckling puppies is not followed by severe illness or death invariably. This is due to variations in the susceptibility depending upon the age, the milk of the dam and on the degree of falls in the post-natal body temperature. Tissue lesions produced by the virus in suckling puppies differ considerably from those usually seen in dogs after weaning. Significance of these findings is discussed.

Acknowledgements

We are grateful to Professor W. I. B. Beveridge and Dr. F. O. MacCallum for their helpful criticism and to our laboratory and animal staff for their unfailing help. Miss M. Lambourne assisted in preparing the manuscript and graphs.

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REVIEWS

CANINE SURGERY, edited by Karl Mayer, J. V. Lacroix and H. Preston Hoskins. Fourth Edition. American Veterinary Publications, Inc., Evanston, Illinois.

THIS valuable and encylopædic work is in its fourth edition and there can be few engaged in this field who have not made its acquaintance. Those few are recommended to do so for there can be no one so experienced that he will not learn much from the study of its contents.

Of course, no reader will agree with all the methods described and many practitioners in this country will consider certain of the techniques over-elaborate and calling for resources beyond his power to command. In those parts of the book where the reader disagrees he will find the comparison of outlook stimulating and perhaps he will be led to re-examine some of the opinions he has held previously.

It is disappointing to find many of the faults of earlier editions uncorrected and it is hoped that the editors will insist on certain changes before a new edition is required—for it is obvious that this will be a standard text on small animal surgery for many years. These faults lie mainly in matters of presentation and style and while variation in the latter is inevitable, and not undesirable, in a work from many hands, there is still far too much flaccid and pompously inflated writing; too often one encounters such absurdities as "cesarotomy" or "emasculation of the testis". And the illustrations: are they really the best that can be obtained? Some appear rather pointless and of the remainder many are poor and quite a few are valueless: figures 339, 438 and 468 for example are surely below the necessary quality and yet they are all of common conditions.

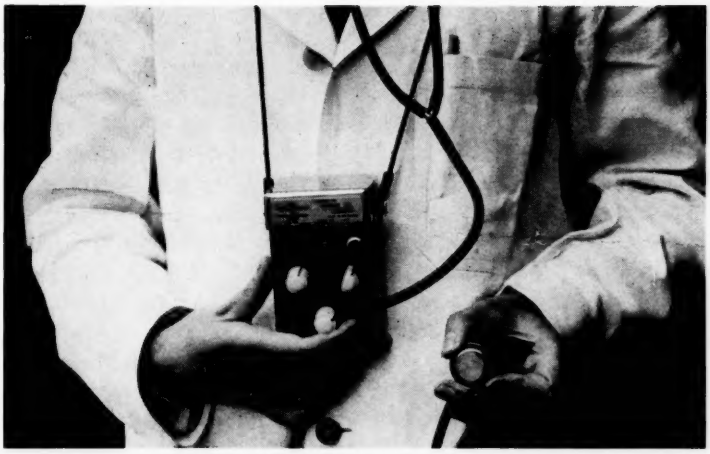
Correction of these faults would require much effort on the part of the editors and perhaps earn them some unpopularity with their contributors but the reviewer believes that the effort and risk would be worth while and could lead to the preparation of a more attractive, shorter and perhaps cheaper book.

SCHÖNBERG ZIETZSCHMANN, Die Ausführung der tierärztlichen Fleischuntersuchung. Fifth Edition. 343 pp. DM. 41.

THIS relatively short textbook of meat inspection is now in its fifth edition from which we may presume that it finds favour in the country of its origin. It is the work of two authors. The late Professor Zietzschmann provides an introductory section on the lymphatic system of the pig, ox and horse which forms an excellent review of this difficult but highly important subject and this chapter can be commended not only to those professionally engaged in the inspection of meat but also to veterinary anatomists.

The larger part, by Professor Schönberg, deals with the examination of the carcase and organs of the ox, small ruminants, pig, horse and dog—the last named animal apparently still finding an occasional place in Continental menus. Each species is considered separately in a series of chapters dealing with the principal diseases of importance in meat inspection and it is interesting to note the relative importance of different conditions in Germany and the United Kingdom: for example, we are reminded once more of the importance of trichinosis on the Continent. Since a foreign work is unlikely to be used as an introductory text it is probable that the aspect that will most interest the British reader is the full and detailed scheme of regulations that govern the decisions of the meat inspector and which apparently leaves little to the discretion of the individual. These regulations are reproduced. Whilst most of the controls appear to be framed wisely it is unlikely that so rigid a system would find acceptance in this country. Also of general interest is the account of meat poisoning and of the bacteriology of the causal organisms, subjects which are treated at greater length than appears appropriate in a professedly elementary work.

ERRATA



An Electronic Stethoscope

British Veterinary Journal Vol. 114, No. 2, 72 - 74

The Editors and Publishers regret that this illustration depicting the electronic stethoscope was omitted when the article by Messrs Price and Stratton was published in the February issue of the British Veterinary Journal.

The general production of the book is of the high standard we now expect from this publishing house.

VETERINARY PROTOZOOLOGY, by U. F. Richardson and S. B. Kendall. Messrs. Oliver and Boyd, Edinburgh and London. 1957. Second, revised edition. Pp. xii + 260. Price 22s. 6d.

THE first edition of Richardson's *Veterinary Protozoology* appeared in 1948 and since then there have been many advances in various aspects of this subject, especially in the field of chemotherapeutic research. Although little progress in some other directions can be recorded there was, nevertheless, the need for a new edition of this textbook. Dr. Kendall has joined Richardson as co-author and their experience in the laboratory and the field in both temperate and tropical countries has enabled them to make a comprehensive revision of the text. The sequence in which the various groups of protozoa are dealt with has been changed and the use of bold type headings is not only pleasing but makes for easier reference to particular parts of the text. Most of the diseases are considered under the headings of: the morphology of the parasite, distribution and transmission, species susceptible, symptoms and pathogenicity, diagnosis, treatment and control. Generally, the text is concise and provides the reader with such factual information as is considered adequate. The practical aspect of the information given is evident from the enlarged final chapter which deals with the techniques and equipment necessary for the diagnosis of protozoal diseases. Some new or revised descriptions of the collection and examination of material, of fixation and staining, of the culture and maintenance of strains of protozoa, of the preparation of media and of serological tests add considerably to the usefulness of the book. Another practical aspect of the text is the detailed information given on the chemistry, pharmacology and use under field conditions of the principal drugs used to control protozoal infections. Special attention has been paid to those developed during the past few years as a result of chemotherapeutic research and trials, used for the treatment of coccidiosis, trypanosomiasis and babesiosis.

Most of the text figures which appeared in the first edition have been retained and to them have been added a few new figures. Amongst the latter, Figure 18 depicting *Eimeria stiedæ* in the bile duct of the rabbit is a poor effort and out of keeping with the other illustrations. There are 207 literature references which have been collected together at the end of the book; in the first edition the relevant references appeared at the end of each section or chapter. In their preface, the authors express the hope that the result of their efforts " will prove equally serviceable as a textbook for the student of veterinary protozoology and as a handbook for the field worker, particularly in tropical countries ". In the reviewer's opinion they need have little anxiety about the realisation of their hopes.

HELMINTHEN UND HELMINTHIASEN DES SCHWEINES, by Curt E. W. Sprehn. Parasitologische Schriftenreihe, Heft 7. Gustav Fischer, Jena, 1957. Price 12.30 Dm. Paper covers.

THE first or general part of this book comprises 14 pages and contains an intro-

duction, an account of the helminths of pigs and their injurious effects, the diagnosis of infections and the general methods of control. The second or special part of the book runs to 120 pages in which the 22 trematodes, nine cestodes, 40 nematodes, one acanthocephalid and two pentastomids that have been reported from pigs are discussed. A morphological description of each species is given as well as its life-cycle, where known, its host range, and geographical distribution. About 30 pages are devoted to accounts of the various helminthiases of pigs such as ascariasis, œsophagostomiasis, fascioliasis, etc. At the end of the book is a key for the determination of the various species mentioned in the text. Following this is a list of the genera and species of pig helminths and their synonyms, 20 pages of approximately 500 literature references and an index. There are 114 illustrations, of which 39 are original, in the text. This is a useful book as it brings together in a concise form a great deal of information about porcine helminths.

NOTICES

The British Caribbean Veterinary Association

THE British Caribbean Veterinary Association is holding its third veterinary convention in Trinidad, B.W.I., from May 25th to 31st, 1958. The first was held in Trinidad in 1954 and the second in Jamaica in 1956.

There will be two days of lectures, two days of surgical demonstration and clinics and one day of films. In addition, there will be an opportunity for excursions to places of interest.

BURROUGHS WELLCOME & CO. announce that "Wellcome" brand Leptospira Canicola Vaccine (Veterinary), for the active immunisation of dogs against Canicola disease, is now issued under the trade mark "Leptovax", in bottles of 3 c.c.

GLAXO LABORATORIES, LTD. announce the introduction of "Predsolan" (prednisolone for the treatment of acetonæmia). It is available in a 10 cc. vial containing 10 milligrams of prednisolone per cc.

The same company announce the introduction of a 500 cc. pack specially designed for multi-dose syringes, of "Saffelin" (a toxoid for the prevention of pulpy kidney disease in sheep). A 50 cc. pack of Saffelin is already on the market.

Veterinary Products in Emergency

BURROUGHS WELLCOME & CO. announce that they have made arrangements whereby veterinary products which are likely to be required in emergency can now be obained from John Bell & Croyden, Ltd., 50-52 Wigmore Street, London,

W.1 (Tel.: WELbeck 5555). This emergency service is limited to weekends and public holidays.

BURROUGHS WELLCOME & Co. announce the introduction of "Frantin"*, for the prevention and treatment of nematodiriasis in lambs. The active ingredient of "Frantin" is Bephenium Embonate, a new anthelmintic discovered in the laboratories of the Wellcome Foundation, Ltd. "Frantin" is available in plastic bottles of 250 gm. (50 doses).

* "Frantin" is a trade mark of the Wellcome Foundation, Ltd.

NEWS

Foot-and-Mouth Disease in Greece

THE Secretary of the FAO European Commission for the Control of Foot-and-Mouth Disease, Dr. E. G. Fogedby, who recently visited Greece, reported as follows:

"Since the last big epizootic in 1951-52, Greece has had many invasions of foot-and-mouth disease. So far the Greek veterinary authorities have been able to limit the outbreaks, and eradicate the disease. The Greek policy is based on strict sanitary measures and ring vaccination in a radius of 10-20 kms., according to conditions. Only in exceptional cases has slaughter of infected and susceptible animals been carried out. In many cases, it is impossible to apply the stamping-out method in a country where on account of common pastures the animal population of a village is as one herd. Compensation by the Government for such large numbers would be prohibitive. There are two main sources of infection; invasion from Turkey and contamination by importation of frozen meat from South American countries.

It is obvious that Turkey is a constant threat to Greece. The two countries have a common border and Greece imports a considerable number of animals for slaughter from Turkey. A great number of Greek outbreaks can be traced to Turkey. The last examples are in connection with the present Turkish epizootic. Greece had been free from foot-and-mouth disease since May 1957. At the beginning of August this year, outbreaks near the Turkish border were reported. The virus causing the outbreak was of type 'O' as in Turkey. The disease spread very rapidly. In one village, for example, about 800 out of 1,000 head of cattle were found to be infected. From the first outbreak, within two weeks the disease spread 200 kms. westward. At the beginning of October, the disease invaded the island of Lamnos, near the Turkish coast. The usual measures were taken, and on the mainland no further spread has occurred since the end of August.

Greece imports annually about 20,000 tons of deep frozen meat, out of which

75 per cent comes from Argentina and Uruguay, and 25 per cent from New Zealand. Five serious outbreaks occurred which undoubtedly could be traced back to the Latin American source. However, control measures taken and strictly applied by the farmers have proved to be very effective. The Greek Government has constructed with its own funds a foot-and-mouth disease laboratory, and substantial help was given by FAO for its equipment. The laboratory will go into production in 1958. The production of culture virus is a complicated procedure, and the veterinary surgeons who are in charge of the laboratory have therefore expressed the hope that FAO will be in a position to give assistance for the commencement of a large-scale production of vaccine so badly needed by the country for a successful campaign against the disease."